THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips, and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The members of the Corporation shall consist of persons elected by the Board of Trustees.

II. The officers of the Corporation shall consist of a President, Vice President, Director, Treasurer, and Clerk.

III. The Annual Meeting of the members shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Massachusetts, at 11:30 A.M., and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the members may be called by the Trustees to be held at such time and place as may be designated.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

VI. Inasmuch as the time and place of the Annual Meeting of members are fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time

and place and purpose of such meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

VII. The Annual Meeting of the Trustees shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Mass., at 10 A.M. Special meetings of the Trustees shall be called by the President, or by any seven Trustees, to be held at such time and place as may be designated, and the Secretary shall give notice thereof by written or printed notice, mailed to each Trustee at his address as shown on the records of the Corporation, at least one (1) week before the meeting. At such special meeting only matters stated in the notice shall be considered. Seven Trustees of those eligible to vote shall constitute a quorum for the transaction of business at any meeting.

VIII. There shall be three groups of Trustees:

(A) Thirty-two Trustees chosen by the Corporation, divided into four classes, each to serve four years; and in addition there shall be two groups of Trustees as follows:

(B) Trustees ex officio, who shall be the President and Vice President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer, and the Clerk;

(C) Trustees Emeriti, who shall be elected from *present* or *former* Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next Annual Meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee *Emeritus* for life. The Trustees *ex officio* and *Emeritus* shall have all the rights of the Trustees except that Trustees *Emeritus* shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

IX. The Trustees shall have the control and management of the affairs of the Corporation; they shall elect a President of the Corporation who shall also be Chairman of the Board of Trustees; and shall also elect a Vice President of the Corporation who shall also be the Vice Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. The Board of Trustees shall have the power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

X. Any person interested in the Laboratory may be elected by the Trustees to a group to be known as Associates of the Marine Biological Laboratory.

XI. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

XII. The account of the Treasurer shall be audited annually by a certified public accountant.

XIII. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen:

The year 1948 from a financial point of view was quite satisfactory. Increased use of the Laboratory's facilities and continued progress in the program of repairs and additions to buildings and equipment were reflected in considerably increased expenditures. The total paid out for current expenses was $264,897.87. The amount spent for additions to capital assets, including $22,856.84 paid on the new boats, was $53,814.81, so that total expenditures were $318,712.68.

Fortunately, current receipts also increased to a total of $291,044.89. To this sum was added $26,539.78 in special, non-recurring receipts that made a total of $317,584.67, so that on a cash basis, the deficit for the year was only $1,128.01. These special receipts consisted of $4,708.11 from the sale of securities given in recent years by Mrs. W. Murray Crane for current expenses and applied with her permission towards the purchase of the "Dolphin," $6,218.88 balance remaining in the Reserve Fund, $9,300.00 given by the American Cancer Society and used chiefly for new equipment, and $6,312.79 balance remaining in the Boat Fund contributed in 1947 and 1948.

The year was also notable for the receipt of 500 shares of Crane Company common stock, valued at $17,250.00 from the Estate of Dr. Frank R. Lillie, and the grant from the Rockefeller Foundation of $250,000., $100,000. for current expenses over a period of five years, and $150,000. for the restoration of "Old Main."

As in recent years, the accounts have been audited by Seamans, Stetson & Tuttle, certified public accountants of Boston, and a copy of their report is available for inspection. The "Balance Sheet" as prepared by them is appended as Exhibit A. As of December 31, 1948, the total *book* value of all the Endowment Assets was $979,865.18, an increase for the year of $1,188.51. The *market* value of these assets was $988,639.62, a reduction for the year of $1,174.54. Plant Assets (Land, Buildings and Equipment) amounted to $1,133,168.64, an increase of $21,217.09.

Following last year's precedent, the "Statements" which follow, prepared with the assistance of Mr. Homer P. Smith, Assistant Business Manager, give the actual financial transactions of the year on a cash basis excluding accruals, non-cash items, depreciation, interdepartment charges, etc. They are:

I. Summary Cash Statement for Year,
II. Current Expenses by Departments,
III. Additions to Capital Assets from Current Funds,
IV. Retirement Fund,
V. Fellowship Fund,
VI. Special Funds,
VII. Real Estate Accounts,
VIII. Agency Accounts.

I. Summary Cash Statement for Year Ended December 31, 1948

	Receipts	Expenditures: Current	Expenditures: Additions to Capital Assets	Expenditures: Total Expenditures
Membership Dues	$ 2,268.00			
Donations for Current Expenses[1]	1,815.00			
Income from Endowment	40,707.09			
Income from Other Securities	23,768.60			
Real Estate Rentals	6,360.00	$ 986.20		$ 986.20
Instruction	12,403.64	8,357.17		8,357.17
Research (incl. Apparatus and Chemical Dep'ts)	25,673.04	26,307.31	$11,346.18	37,653.49
Mess	38,858.06	39,375.21	417.13	39,792.34
Dormitories and Apt. House	16,788.30	14,175.34	327.71	14,503.05
Library[2]	5,989.85	9,431.37	7,983.41	17,414.78
Buildings and Grounds		42,276.09	4,367.57	46,643.66
Supply Department[3]	106,724.12	85,693.13	29,372.81	115,065.94
"Biological Bulletin"	7,538.15	8,345.89		8,345.89
Administration		27,652.96		27,652.96
Miscellaneous	2,151.04	2,297.20		2,297.20
	$291,044.89	$264,897.87	$53,814.81	$318,712.68
Special Receipts				
Sale of Securities $4,708.11				
Reserve Fund 6,218.88				
American Cancer Soc. 9,300.00				
Boat Fund 6,312.79	26,539.78			
	$317,584.67			

Total Expenditures	$318,712.68
Total Receipts	317,584.67
Cash Deficit for Year	$ 1,128.01

Notes:

[1] Donations were $1,320. given by the "Associates" of the Laboratory and $495. miscellaneous contributions.

[2] The Library income consists of $3,000. payment from the Oceanographic Institution towards Library expenses and $2,989.85 from the Carnegie Book Fund (balance of Book Fund still available is $11,416.15). The monetary value of serials received in exchange for the "Bulletin," estimated at $3,571.26, is not included, nor is the $800. received from the Oceanographic Institution for the purchase of books for their account.

[3] The actual 1948 sales of the Supply Department were $94,701.77, a slight decrease from the 1947 sales. The values of specimens and supplies furnished the Research and Instruction Departments were $8,817.50 and $5,488.82 respectively. If these values are taken into account, and also the gain in inventory of $6,042.36, the decrease in accounts receivable of $14,101.28, and a debit charge of $2,100. for administration and maintenance expense, there would be a net profit of $25,178.39 on the operations of the Supply Department for 1948. This does not take into account the $6,515.97 spent for capital items, exclusive of the new boats, or the auditors' charges of $1,684.38 for depreciation. If these had been included, the net profit for the Supply Department would have been $16,978.04.

II. Current Expenses for 1948 by Departments

Administration

Salaries		$ 21,661.02
Central Hanover Bank Trustee Commissions		1,036.22
Falmouth Bank Charges		154.53
Audit		1,162.35
Treasurer's Office		600.00
Advertising		374.52
Office Supplies		1,320.08
Sundries (Tel., Postage, etc.)		1,803.82
		28,112.54
Deduct Cash Receipts		459.58
		27,652.96

Instruction

Salaries and Travel		8,210.40
Sundries		146.77
		8,357.17

Research (incl. Apparatus and Chemical Dep'ts)

Salaries		14,316.12
Travel		200.00
Repairs		344.66
Supplies and Sundries		13,245.80
		28,106.58
Deduct Cash Receipts		1,799.27
		26,307.31

Library

Salaries		8,947.57
Office Supplies		190.08
Sundries		333.38
		9,471.03
Deduct Cash Receipts		39.66
		9,431.37

Buildings and Grounds

Salaries and Wages		25,253.60
Fuel		3,711.08
Gas		1,557.45
Light and Power		3,320.66
Water		722.29
Insurance		1,706.71
Repairs		4,329.09
Sundries		2,043.13
		42,644.01
Deduct Cash Receipts		367.92
		42,276.09

Dormitories and Apt. House

Salaries and Wages		5,576.27
Lighting, Gas and Water		2,114.74
Repairs		3,082.81
Outside Rentals		500.00
Laundry		1,455.41
Insurance		762.22
Sundries		716.17
		14,207.62
Deduct Cash Receipts		32.28
		$ 14,175.34

Mess

Salaries and Wages		$ 8,800.84
Cost of Food		25,989.86
Gas, Water, Light and Power		2,151.89
Repairs		89.48
Replacements of Dishes, etc.		647.78
Insurance		623.98
Laundry		327.23
Freight and Express		58.08
Sundries		780.16
		39,469.30
Deduct Cash Receipts		94.09
		39,375.21

Supply Department

Salaries and Wages		33,607.64
Purchase of Specimens		32,529.14
Chemicals		2,384.83
Containers		3,826.05
Boat Expenses		2,631.38
Truck Expenses		559.94
Freight and Express		3,107.31
Fuel, Light and Power		809.35
Office Supplies		460.08
Telephone and Telegraph		206.29
Insurance (incl. Boats)		2,381.84
Advertising		392.76
Specimens and Supplies purchased for Research Dep't		1,035.64
Nets, Floats, etc.		461.00
Repairs and Sundries		1,349.08
		85,742.33
Deduct Cash Receipts		49.20
		85,693.13

"Biological Bulletin"

Salaries	2,130.00
Printing, etc.	6,222.84
	8,352.84
Deduct Cash Receipts	6.95
	8,345.89

Real Estate (Leased Out)

Taxes and Insurance on Bar Neck Property (Garage) and Janitor's House	986.20

Other Expenses

Workmen's Compensation Insurance	1,004.67
Truck Expense	462.99
Bay Shore and Great Cedar Swamp Expenses	256.47
Interest on Mortgage	406.67
Evening Lectures	166.40
	2,297.20
Total Expenses	$264,897.87

III. Additions to Capital Assets from Current Funds

A. Buildings

Brick Laboratory	$ 2,247.59
Supply Dep't	6,318.12
Mess	202.27
	$ 8,767.98

B. Library

Back Sets	$ 1,903.37
Books	569.15
Serials	2,989.39
Reprints	13.75
Binding	2,507.75
	$ 7,983.41

C. Equipment

Apparatus Dep't	$ 9,069.71
Dormitories	327.71
Mess	214.86
Brick Laboratory	306.97
Carpenter Shop	421.42
Boats	22,856.84
Supply Dep't	197.85
Truck	1,391.59
Machine Shop	2,276.47
	$37,063.42
Total Additions to Capital Assets	$53,814.81

IV. Retirement Fund (Securities and Cash)

Jan. 1, 1948, Balance on Hand		$16,388.44
Receipts:		
Income and Principal Gains	$ 243.02	
Payment from M.B.L. (10% of 1948 Payroll)	7,323.62	7,566.64
		23,955.08
Disbursements:		
Pensions paid in 1948	5,710.00	
Custodian Fees	50.00	5,760.00
Dec. 31, 1948, Balance on Hand		$18,195.08

V. Fellowship Fund

Jan. 1, 1948, Balance on Hand		$ 1,043.03
Receipts:		
Payment from Lalor Foundation	$ 5,000.00	5,000.00
		6,043.03
Disbursements:		
Fellowships (nine)	3,665.35	
Laboratory Space, Apparatus and Supplies	1,499.53	5,164.88
Dec. 31, 1948, Balance on Hand		$ 878.15

VI. Special Funds

A. Boat Fund

Jan. 1, 1948, Balance on Hand		$ 5,302.79
Receipts:		
Contributions	$ 1,010.00	1,010.00
		6,312.79
Disbursements:		
Transfer to M.B.L. Income Account for New Boats		6,312.79
		-0-

B. Dr. Frank R. Lillie Memorial Fund

Jan. 1, 1948, Balance on Hand (Initial Contribution from Dr. G. H. A. Clowes)	$ 1,000.02
No Transactions in 1948	

VII. Real Estate Accounts

A. Devil's Lane Property

Cash Received in 1948 from Sales	$ 4,097.50
Disbursements (Taxes)	193.30
Book Value of Unsold Lots, Dec. 31, 1948	36,739.43
Accounts Receivable, Dec. 31, 1948	6,662.00

B. Gansett Property

Cash Received in 1948	$ 845.00
Disbursements (Taxes)	85.44

All Gansett lots having been sold, cash balance of $522.20 representing net profit on sale of last five lots was transferred to Current Income account, and Gansett account was closed.

VIII. Agency Accounts

A. Cancer Research Account

(U. S. Public Health Service Project under direction of Dr. Robert Chambers)

Jan. 1, 1948, Balance on Hand		$18,721.19
Disbursements:		
Payments for Salaries, Laboratory Space, Apparatus and Supplies	$18,719.96	
Balance Remitted to the United States Treasury	1.23	18,721.19
		-0-

EXHIBIT A

Marine Biological Laboratory Balance Sheet, Dec. 31, 1948

(From Auditors' Report)

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee		$ 961,729.66	
Securities and Cash in Minor Funds		18,135.52	$ 979,865.18
Plant Assets:			
Land	$ 110,626.38		
Buildings	1,345,956.86		
Equipment	238,252.31		
Library	374,880.39		
	$2,069,715.94		
Less Reserve for Depreciation	747,963.45	$1,321,752.49	
Book Fund, Securities and Cash		11,416.15	$1,333,168.64
Current Assets:			
Cash		$ 15,257.21	
Mortgage Note Receivable		2,350.00	
Accounts Receivable		24,420.03	
Inventories:			
Supply Department	$ 49,974.46		
"Biological Bulletin"	16,195.99	66,170.45	
Investments:			
Devil's Lane Property	43,401.43		
Stock in General Biological Supply House, Inc.	12,700.00		
Other Investment Securities	35,020.00		
Retirement Fund	18,195.08	109,316.51	
Prepaid Insurance		5,959.57	
Items in Suspense (Debits)		2,092.72	$ 225,566.49
			$2,538,600.31

Liabilities

Endowment Funds:			
Endowment Funds	$ 960,016.27		
Reserve for Amortization	1,713.39	$ 961,729.66	
Minor Funds		18,135.52	$ 979,865.18
Plant Funds:			
Mortgage Notes Payable		$ 10,000.00	
Donations and Gifts	$1,172,564.04		
Other Investments in Plant from Gifts and Current Funds	150,604.60	1,323,168.64	$1,333,168.64
Current Liabilities and Surplus:			
Accounts Payable		$ 13,552.25	
Items in Suspense (Credits)		2,582.07	
Current Surplus		209,432.17	$ 225,566.49
			$2,538,600.31

Respectfully submitted,

Donald M. Brodie,
Treasurer

V. REPORT OF THE ACTING LIBRARIAN

1948

In 1948 the Library staff carried on the necessary routine activities and continued with projects already in progress.

The sum of $11,500 was appropriated to the Library, plus $3000 from the Woods Hole Oceanographic Institution, a contribution toward the salaries of the staff. For a detailed account of the expenditures of the budget, $13,077.73, reference may be made to the Treasurer's report. The sum of $800 was provided by the Woods Hole Oceanographic Institution for library acquisitions.

During the year, 1209 (55 new) current journals were received. Of these, 329 (12 new) were Marine Biological Laboratory subscriptions, 510 (14 new) were exchanges, and 168 (3 new) were gifts; 51 (4 new) were Woods Hole Oceanographic Institution subscriptions, 137 (17 new) were exchanges, and 14 (5 new) were gifts. Due to the great irregularity in current receipts of German and Russian journals, most of these titles were omitted in this tabulation.

The Marine Biological Laboratory purchased 78 books, received 9 complimentary copies from authors, 43 gifts from the publishing firms, 152 books from the E. L. Mark Library, and 23 miscellaneous donations. The Woods Hole Oceanographic Institution purchased 32 books. These made a total of 337 titles acquired.

There were 27 back sets completed: 18 by purchase (5 Woods Hole Oceanographic Institution), 6 by exchange (3 Woods Hole Oceanographic Institution), and 3 by gift (2 Woods Hole Oceanographic Institution); 55 were partially completed: 39 by purchase (9 Woods Hole Oceanographic Institution), 1 by exchange (Woods Hole Oceanographic Institution), and 15 by gift.

The reprint additions to the Library numbered 11,310. Of these, 1385 were of current issue and the others were of earlier dates. Of the 24,690 papers acquired through the collections of Drs. Ulrich Dahlgren, Alfred C. Redfield, Frank R. Lillie, and a gift from the Boston University School of Medicine, 4642 were found to be reprints not already appearing in the Library's collection.

A sum of $2989.85 from the Carnegie Corporation of New York Fund was spent for 6 books, 9 completed back sets, 16 partially completed back sets, and the binding of 337 volumes.

There were 86 microfilm orders filled during the year. Libraries and individuals have been encouraged to use this service in preference to requesting volumes on inter-library loan. In spite of this effort, 94 volumes were sent out on loan, and 51 were borrowed for the investigators.

Several valuable gifts were received in 1948. The most outstanding of these was the collection of Dr. Frank R. Lillie's reprints. Dr. Lillie's thought in requesting that this Library inherit his collection was one of the many kindnesses shown throughout the years of his association with this Laboratory. Grateful acknowledgment is also made to the Bermuda Biological Station for Research, Inc., for the gift of the E. L. Mark Library of books and reprints. Time has not permitted the complete assorting of this huge amount of material, but 152 books and 1600 reprints have been added to the shelves. Among these were several very valuable books by Louis Agassiz. The Library is also indebted to the American

Academy of Arts and Sciences for a four-volume set of Count Rumford's works.

The Library contained at the end of 1948, 57,548 bound volumes and 160,528 reprints.

Respectfully submitted,

DEBORAH LAWRENCE
Acting Librarian

VI. THE REPORT OF THE DIRECTOR

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY

Gentlemen:

I submit herewith a report of the sixty-first session of the Marine Biological Laboratory for the year 1948.

During the past year our physical plant has undergone many changes, chief of which are the renovation of Old Main; the purchase of the Elliott House; the purchase of the Dolphin; and the development of a laboratory for work with radioactive isotopes.

1. *Renovation of Old Main*

The generous gift of $150,000 made by the Rockefeller Foundation for the renovation of Old Main has enabled us to make necessary and long desired changes in that building. Preliminary plans for the work were prepared by the firm of Coolidge, Shepley, Bulfinch, and Abbott in consultation with the Building Committee consisting of Mr. Claff, Chairman, and Drs. Marsland, Parpart, and Packard. This Committee was later enlarged to include Messrs. MacNaught, Smith, Robert Kahler, and Pierce of the Laboratory Staff. Because of the high cost of construction not all of the changes desired by the Committee could be made. However, with the available funds, the building, which is structurally sound, can be put into excellent condition.

It was decided to construct a basement under the entire building during the Spring of 1949, and to finish the renovation of the upper floors after the close of the summer session. By this arrangement, the classes can be held as usual, without any interruption. The Spring operations are in charge of the Sawyer Construction Company which built the new wing of the Library. The basement contains ten research rooms, two general laboratories, all equipped with sea water tables, and other necessary facilities, three dark rooms with sea water tables, a cold room (to be completed later), and service rooms. Between the wings of the building a sunken court provides light for the rooms opening on to it, and space for two large fish pools. The old plumbing, for many years in deplorable condition, has been completely replaced in the basement, and will be greatly improved elsewhere after the close of the summer season. Additions to the northwest and southwest corners of the building give extra space in the basement and on the first and second floors. The total area of the basement plus these newly constructed rooms is about equal to one floor of the Crane Building. Thanks to the Rockefeller Foundation we shall have a laboratory which will adequately accommodate the classes and the instructors for many years.

Some of our workers have doubted the wisdom of spending $150,000 on an old building, while others are pleased that Old Main, where so much important biological research has been carried on during the past sixty years, will be used by new generations of students. While a completely new structure would be desirable, there seemed to be no prospect of obtaining funds for the construction and equipment. On the other hand, the old building is well worth preserving. Mr. Sawyer, under whose direction the renovation has been made, writes that "The existing walls, floors and roof construction in Old Main are in excellent condition, although certain parts of the wall and roof shingling must be renewed in the comparatively near future. In our opinion, you have done the economical thing in making use of as much of Old Main as is possible."

2. *The Elliott House*

As a result of the purchase of the Elliott House the Laboratory now owns all the property on both sides of Center Street. This house, which will accommodate about twenty people, has been improved by the addition of an upstairs bathroom, and other changes.

3. *The Dolphin*

The purchase of a new boat, in addition to the *Limulus*, was mentioned in the last Annual Report. The new craft, the *Dolphin*, was in service during the summer of 1948, taking the classes on field trips and making collections of living material. It accommodates seventy passengers, is seaworthy and fast, and is a welcome addition to the fleet. Its gasoline engine has now been replaced by a Diesel motor acquired from the War Assets Administration and installed by our machinist, Mr. Harlow.

4. *The Radiation Laboratory*

Under the direction of Dr. Failla, the Radiation Laboratory has been greatly expanded. The American Cancer Society last year contributed $4,300 for the purchase of measuring instruments and other apparatus, and for equipment needed to safeguard investigators using radio-active substances. A "hot laboratory" where these materials can be transferred is now furnished with special tables, cabinets, and a hood.

This new field of research has attracted many workers. During the summer of 1948 fifteen investigators were using radio-active isotopes, among them being four Fellows supported by funds granted by the Atomic Energy Commission.

To carry on this type of research successfully, investigators require special training. For this reason Dr. Failla arranged a course of lectures given by well known specialists. Dr. Paul Aebersold, Chief of the Isotopes Division at Oak Ridge, took an active part in the course, and was available for advice on technical problems.

5. *Winter Research*

During a large part of the year the Institute of Muscle Research, under the direction of Dr. Szent-Gyorgyi has carried on research here. The number of investigators has grown until now there are eight at work. The laboratory of Experi-

mental Cell Research, directed by Dr. Chambers, occupied rooms during the winter of 1947–48, but has now moved to New York City.

6. *Gifts*

The Rockefeller Foundation, in addition to its gift of $150,000 for the renovation of Old Main, has also granted for the general support of the Laboratory the sum of $100,000, available during the next five years at the rate of not more than $25,000 per year. This is most welcome, for we stand in need of funds to replace old equipment, especially in the Apparatus and Chemical Departments, and to purchase other apparatus required for research along lines not heretofore followed at this Laboratory. This gift, however, is not restricted to such purposes and can be used for other laboratory needs.

In his will, Dr. F. R. Lillie bequeathed to the Laboratory 500 shares of Crane Company stock, the book value of which is $17,250.

Other gifts have been received from

The Schwarzhaupt Foundation towards the purchase of a new boat	$1,000
Dr. W. D. Curtis, for a bandsaw in the Carpenter Shop	$225
Mr. Leo H. Spivack	$200
The M. B. L. Associates	$1,320
The American Cancer Society to purchase apparatus to be used in the Laboratory of Experimental Cell Research and for equipment and technical help in the Radiation Laboratory	$5,000 $4,300

7. *Changes in Personnel*

Dr. E. P. Little, manager of the Apparatus Department since 1942, and of the Chemical Department during the war years, has resigned in order to carry on research at the Computation Laboratory of Harvard University.

During his incumbency he greatly enlarged the Apparatus Department and increased its efficiency. We are glad to retain him as a Consultant. The new manager is Mr. Robert Mills who has been associated with Dr. Little for some time. Mr. Gail Cavanaugh, head of the Science Department at the Falmouth High School, has been appointed manager of the Chemical Department.

We shall miss "Colonel" Wamsley who died in the spring of 1949. Coming here in 1892 as a student from Brown University he became a member of the Corporation. He was connected with the Charleston, S. C. Museum for many years, and served as a superintendent of one of the city schools. During the summers he was a special collector in the Supply Department.

8. *Election of Trustees*

At the meeting of the Corporation, August 10, 1948, the following trustees were elected.

Class of 1952

E. S. G. Barron
D. W. Bronk
G. Failla
C. O'D. Iselin
R. T. Kempton
C. W. Metz
Wm. Randolph Taylor
George Wald

Albert Tyler was elected to fill the vacancy caused by the death of S. C. Brooks of the Class of 1949.

H. H. Plough was elected in place of G. H. A. Clowes of the Class of 1951, who was made Trustee Emeritus.

9. *There are appended as parts of this report:*

1. The Staff
2. Investigators and Students
3. The Lalor Fellows
4. The Atomic Energy Commission Fellows
5. Tabular View of Attendance
6. Subscribing and Cooperating Institutions
7. Evening Lectures
8. Shorter Scientific Papers Presented at the Seminar
9. Members of the Corporation

Respectfully submitted,
CHARLES PACKARD,
Director

1. THE STAFF, 1948

CHARLES PACKARD, Director, Marine Biological Laboratory, Woods Hole, Massachusetts.

SENIOR STAFF OF INVESTIGATION

E. G. CONKLIN, Professor of Zoology, Emeritus, Princeton University.
RALPH S. LILLIE, Professor of General Physiology, Emeritus, The University of Chicago.
A. P. MATHEWS, Professor of Biochemistry, Emeritus, University of Cincinnati.
G. H. PARKER, Professor of Zoology, Emeritus, Harvard University.

ZOOLOGY

I. CONSULTANTS

LIBBIE H. HYMAN, American Museum of Natural History.
A. C. REDFIELD, Woods Hole Oceanographic Institution.

II. INSTRUCTORS

F. A. BROWN, Professor of Zoology, Northwestern University, in charge of course.
W. D. BURBANCK, Professor of Biology, Drury College.
C. G. GOODCHILD, Professor of Biology, S.W. Missouri State College.
L. H. KLEINHOLZ, Associate Professor, Reed College.
JOHN H. LOCHHEAD, Assistant Professor of Zoology, University of Vermont.
MADELENE E. PIERCE, Associate Professor of Zoology, Vassar College.
W. M. REID, Professor of Biology, Monmouth College.
T. H. WATERMAN, Assistant Professor in Biology, Yale University.

III. LABORATORY ASSISTANTS

R. S. HOWARD, University of Miami.
MARIE WILSON, Northwestern University.

EMBRYOLOGY

I. Consultants

H. B. Goodrich, Professor of Biology, Wesleyan University.
Albert Tyler, Associate Professor of Embryology, California Institute of Technology.

II. Instructors

Donald P. Costello, Prfoessor of Zoology, University of North Carolina, in charge of course.
Arthur L. Colwin, Assistant Professor of Zoology, Queens College.
Howard L. Hamilton, Associate Professor of Zoology, Iowa State College.
Charles B. Metz, Assistant Professor of Zoology, Yale University.

III. Research Assistant

Marjorie Hopkins Fox, University of California.

IV. Laboratory Assistant

Helen A. Padykula, Mount Holyoke College.

PHYSIOLOGY

I. Consultants

Eric G. Ball, Professor of Biochemistry, Harvard University Medical School.
Merkel H. Jacops, Professor of Physiology, University of Pennsylvania.
Otto Loewi, Professor of Pharmacology, New York University, School of Medicine.
Arthur K. Parpart, Professor of Biology, Princeton University.

II. Instructors

E. S. Guzman Barron, Associate Professor of Biochemistry, The University of Chicago, in charge of course.
M. J. Kopac, Associate Professor of Biology, New York University.
John F. Muntz, Assistant Professor of Biochemistry, Western Reserve University Medical School.
Robert F. Pitts, Professor of Physiology, Syracuse University, College of Medicine.
H. Burr Steinbach, Professor of Zoology, University of Minnesota.
George Wald, Professor of Biology, Harvard University.
Dorothy Wrinch, Lecturer, Smith College.

BOTANY

I. Consultants

P. R. Burkholder, Eaton Professor of Botany, Yale University.
W. R. Taylor, University of Michigan.

II. Instructors

Maxwell S. Doty, Assistant Professor of Botany, Northwestern University. In Charge of Course.
H. C. Bold, Vanderbilt University.
R. D. Northcraft, Rutgers University.

III. Research Assistants

Justine Garnic, Carnegie Institute of Technology.
Leonard E. Spiegel, Drew University.

IV. Laboratory Assistant

Remi J. Cadoret, Harvard University.

V. Lecturers

J. B. Lackey, Philadelphia, Pa.
R. D. Wood, Rhode Island State College.

VI. Field Consultant and Collector

Edwin T. Moul, University of Pennsylvania.

EXPERIMENTAL RADIOLOGY

G. Failla, College of Physicians and Surgeons, Columbia University.
L. Robinson Hyde, Phillips Exeter Academy, Exeter, N. H.

LIBRARY

Deborah Lawrence, Acting Librarian
Margaret P. McInnis — Mary A. Rohan
Jean Goodfellow

APPARATUS DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager
J. D. Graham — Dorothy LeFevre
Robert Mills

CHEMICAL DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager

SUPPLY DEPARTMENT

James McInnis, Manager
John S. Rankin, Naturalist
Ruth Crowell — Marcia McLaughlin
M. B. Gray — W. E. Kahler — R. E. Tonks
A. M. Hilton — W. S. Landers — F. N. Whitman
G. Lehy

GENERAL OFFICE

F. M. MacNaught, Business Manager
Homer P. Smith, Assistant Business Manager
Polly L. Crowell — Mrs. Lila S. Myers

GENERAL MAINTENANCE

R. W. Kahler, Manager

Robert Adams — A. Neal
R. Gunning — G. T. Nichelson, Jr.
J. H. Head — A. J. Pierce
G. A. Kahler — T. E. Tawell
Seaver R. Harlow

THE GEORGE M. GRAY MUSEUM

2. Investigators and Students

Independent Investigators, 1948

Abelson, Philip H., Staff Member, Carnegie Institute of Washington.
Abrams, Richard, Assistant Professor, University of Chicago.
Aebersold, Paul C., Chief, Isotopes Branch, Atomic Energy Commission.
Agersborg, H. P. K., Professor of Histology, Des Moines Still College.
Allen, M. Jean, Instructor in Biology, Mather College.
Amberson, William R., Professor of Physiology, University of Maryland Medical School.
Ardao, Maria Isabel, University of Montevideo.
Armaghan, Veronica, New York City.
Armstrong, Philip B., Professor of Anatomy, College of Medicine, Syracuse University.
Augustinsson, Klas-Bertil, University of Stockholm.
Baker, Gladys E., Associate Professor of Plant Science, Vassar College.
Baldwin, Ernest H. F., University Lecturer in Biochemistry, Cambridge, England.
Ball, Eric G., Professor of Biological Chemistry, Harvard Medical School.
Barron, E. S. Guzman, Associate Professor of Biochemistry, University of Chicago.
Berger, Charles A., Director, Biological Laboratory, Fordham University.
Bliss, Alfred F., Assistant Professor of Physiology, Tufts Medical School.
Blum, Harold F., Physiologist, National Cancer Institute.
Bonner, John T., Assistant Professor of Biology, Princeton University.
Bowers, John Z., Assistant and Director and Chief, U. S. Atomic Energy Commission.
Boyer, Donald D., Instructor in Biology, Union College.
Bridgman, Anna J., Professor of Biology, Limestone College.
Brooks, Matilda, Professor of Zoology, University of California.
Brown, Frank A., Jr., Professor of Zoology, Northwestern University.
Burbanck, William D., Professor of Biology, Drury College.
Butler, Elmer G., Professor of Zoology, Princeton University.
Cameron, Gladys, Research Associate, New York City.
Cannan, R. Keith, Professor of Chemistry, New York University College of Medicine.
Chambers, Robert, Chief, Laboratory of Experimental Cell Research.
Chance, Britton, Assistant Professor of Biophysics, Johnson Research Foundation.
Chase, Aurin M., Associate Professor of Biology, Princeton University.
Cheney, Ralph H., Associate Professor of Biology, Brooklyn College.
Chrystall, Frieda L., Teacher of Biology, Julia Richman High School.
Chu, Hao-jan, Graduate Assistant in Botany, Northwestern University.
Claff, C. Lloyd, Research Fellow in Surgery, Harvard Medical School.
Clark, Arnold M., Instructor in Biology, University of Delaware.
Clark, Eliot R., Professor of Anatomy, University of Pennsylvania School of Medicine.
Clark, Leonard B., Professor of Biology, Union College.
Claude, Albert, Associate Member, The Rockefeller Institute.
Clement, A. C., Professor of Biology, College of Charleston.
Clowes, G. H. A., Research Director Emeritus, Lilly Research Laboratories.
Cohen, Isadore, Assistant Professor, American International College.
Cole, Kenneth S., Professor of Biophysics, University of Chicago.
Colwin, Arthur L., Assistant Professor of Biology, Queens College.
Conklin, Edwin G., Professor of Biology Emeritus, Princeton University.
Cooper, Kenneth W., Associate Professor of Biology, Princeton University.
Copeland, E. Eugene, Assistant Professor of Biology, Brown University.
Cornman, Ivor, Research Fellow, Sloan-Kettering Institute.
Costello, Donald P., Professor of Zoology, University of North Carolina.
Coyle, Elizabeth E., Assistant Professor of Biology, College of Wooster.
Crowell, Sears, Associate Professor of Zoology, Miami University.
Dent, J. N., Assistant Professor, University of Pittsburgh.
Diller, Irene C., Research Cytologist, Institute for Cancer Research, Lankinau Hospital.

DILLER, WILLIAM F., Assistant Professor of Zoology, University of Pennsylvania.
DOTY, MAXWELL S., Assistant Professor of Botany, Northwestern University.
DURYEE, WILLIAM R., Research Associate, Carnegie Institution of Washington.
EDGERLEY, ROBERT H., Research Scientist, Columbia University.
EICHEL, BERTRAM, Research Associate, New York University College of Dentistry.
EVANS, TITUS C., Research Professor of Radiology, State University of Iowa.
FAHEY, ELIZABETH M., Teacher, Taunton High School.
FAILLA, G., Professor of Radiology, Columbia University.
FLOOD, VERONICA M., Junior Scientist, Argonne National Laboratories.
FRENKEL, ALBERT W., Assistant Professor of Botany, University of Minnesota.
FROEHLICH, ALFRED, Associate, The May Institute for Medical Research.
GAFFRON, HANS, Associate Professor of Biochemistry, University of Chicago.
GERARD, POL, Professor of Pathology, The Belgian American Educational Foundation.
GLASER, O. C., Professor of Biology, Amherst College.
GOLDSTEIN, AVRAM, Instructor in Pharmacology, Harvard University Medical School.
GOODCHILD, C. G., Professor of Biology, Missouri State College.
GOULD, HARLEY N., Professor of Biology, Tulane University, Newcomb College.
GRAND, C. G., Research Associate, New York University.
GRAY, IRVING M., Professor of Zoology, Duke University.
GREENBERG, G. ROBERT, Senior Instructor in Biochemistry, Western Reserve University.
GREGG, JOHN R., Assistant Professor of Zoology, Columbia University.
GROSCH, DANIEL S., Assistant Professor of Zoology, North Carolina State College.
GRUNDFEST, HARRY, Assistant Professor of Neurology, Columbia University.
HAMILTON, HOWARD L., Associate Professor of Zoology, Iowa State College.
HARVEY, E. NEWTON, Professor of Physiology, Princeton University.
HARVEY, ETHEL BROWNE, Independent Investigator, Princeton University.
HAYWOOD, CHARLOTTE, Professor of Physiology, Mount Holyoke College.
HEIDENTHAL, GERTRUDE, Assistant Professor of Biology, Russell Sage College.
HEILBRUNN, L. V., Professor of Zoology, University of Pennsylvania.
HESTRIN, SHLOMO, Research Assistant, College of Physicians and Surgeons.
HOPKINS, HOYT S., Associate Professor of Physiology, New York University College of Dentistry.
HSIAO, SIDNEY CO., Guest Professor of Biology, Osborn Zoological Laboratory.
HUNTER, FRANCIS R., Associate Professor of Zoology, University of Oklahoma.
HUTCHENS, JOHN O., Chairman, Department of Physiology, University of Chicago.
JACOBS, M. H., Professor of General Physiology, University of Pennsylvania.
JANNEY, CLINTON D., Research Assistant Professor of Physiology, State University of Iowa.
JENKINS, GEORGE B., Professor of Anatomy Emeritus, George Washington University.
KABAT, ELVIN A., Assistant Professor of Bacteriology, College of Physicians and Surgeons.
KARUSH, FRED, Fellow, New York University College of Medicine.
KELLER, RUDOLPH, Director of Biochemical Research, Madison Foundation.
KEMPTON, RUDOLF T., Professor of Zoology, Vassar College.
KISCH, BRUNO, Professor, Yeshiva University.
KITCHIN, IRWIN C., Associate Professor of Biology, University of Georgia.
KLEINHOLZ, LEWIS H., Associate Professor of Biology, Reed College.
KLOTZ, IRVING M., Associate Professor of Chemistry, Northwestern University.
KOPAC, M. J., Associate Professor of Biology, New York University.
KRAHL, MAURICE E., Assistant Professor of Pharmacology, Washington University.
LAZAROW, ARNOLD, Assistant Professor of Anatomy, Western Reserve University.
LEFEVRE, PAUL G., Assistant Professor of Physiology, University of Vermont.
LEIN, JOSEPH, Instructor in Biology, Syracuse University.
LEVY, MILTON, Associate Professor of Chemistry, New York University College of Medicine.
LIBET, BENJAMIN, Assistant Professor of Physiology, University of Chicago.
LILLIE, RALPH S., Professor of Physiology Emeritus, University of Chicago.
LIU, CHIEN KANG, Research Fellow, Laboratory of Experimental Cell Research.
LOCHHEAD, JOHN H., Assistant Professor of Zoology, University of Vermont.
LOVELACE, ROBERTA, Adjunct Professor of Biology, University of South Carolina.
LUCKE, BALDUIN, Professor of Pathology, University of Pennsylvania.

MARSHAK, ALFRED, Research Associate, New York University Medical College.
MARSLAND, DOUGLAS A., Professor of Biology, New York University, Washington Square College.
MAZIA, DANIEL, Professor of Zoology, University of Missouri.
METZ, CHARLES B., Assistant Professor of Zoology, Yale University.
MICHALSKI, JOSEPH V., Assistant Professor of Anatomy, Emory University.
MILLER, JAMES A., Associate Professor of Anatomy, Emory University.
MUNTZ, JOHN A., Assistant Professor, Western Reserve University.
NACHMANSOHN, DAVID, Assistant Professor of Neurology, College of Physicians and Surgeons.
NAUSS, MRS. RALPH, 1303 York Avenue, New York City.
NELSON, LEONARD, Teaching Assistant in Zoology, University of Minnesota.
NEWFANG, DOROTHY M., Assistant Professor of Botany, Elmira College.
NORTHROP, JOHN H., Head of Department of General Physiology, Rockefeller Institute.
NUTTING, WILLIAM B., Instructor in Zoology, University of Massachusetts.
O'BRIEN, JOHN A., Assistant Professor of Biology, Catholic University of America.
OLSON, MAGNUS, Assistant Professor of Zoology, University of Minnesota.
OSTER, ROBERT H., Professor of Physiology, University of Maryland.
OSTERHOUT, W. J. V., Member Emeritus, Rockefeller Institute.
PALAY, SANFORD L., Visiting Investigator, Rockefeller Institute.
PARMENTER, C. L., Professor of Zoology, University of Pennsylvania.
PARPART, ARTHUR K., Professor of Biology, Princeton University.
PARSHLEY, H. M., Professor of Zoology, Smith College.
PASTEELS, JEAN J., Professor of Anatomy, University of Brussels.
PERSKY, HAROLD, Director of Research, Michael Reese Hospital.
PICK, JOSEPH, Associate Professor of Anatomy, New York University College of Medicine.
PIERCE, MADELENE E., Associate Professor of Zoology, Vassar College.
PITTS, ROBERT F., Professor of Physiology, Syracuse University College of Medicine.
POCOCK, MARY A., Senior Lecturer in Botany, Rhodes University, South Africa.
PRAT, HENRI, Professor, University of Montreal.
PRICE, WINSTON H., Special Investigator, Rockefeller Institute.
PROSSER, C. LADD, Associate Professor of Zoology, University of Illinois.
RAUT, CAROLINE, Assistant Professor, Southern Illinois University.
REID, W. MALCOLM, Professor of Biology, Monmouth College, Monmouth, Illinois.
ROSE, S. MERYL, Associate Professor of Zoology, Smith College.
ROSSI, HAROLD H., Physicist, Department of Radiology, Columbia University.
RUDZINSKI, MARIE A., Instructor, New York University, Washington Square College.
RUGH, ROBERTS, Associate Professor of Biology, New York University.
SCHAEFFER, A. A., Professor of Biology, Temple University.
SCHMIDT, GERHARD, Senior Research Associate, Tufts College Medical School.
SCOTT, ALLAN C., Associate Professor of Biology, Union College.
SCOTT, SISTER FLORENCE MARIE, Professor of Biology, Seton Hill College.
SCOTT, GEORGE T., Assistant Professor of Biology, Oberlin College.
SHANES, ABRAHAM M., Associate Professor of Physiology, Georgetown University School of Medicine.
SICHEL, F. J., Professor of Physiology, University of Vermont.
SLIFER, ELEANOR H., Assistant Professor of Zoology, State University of Iowa.
SPEIDEL, CARL C., Professor of Anatomy, University of Virginia.
STEKLER, BURTON L., Student, New York University College of Medicine.
STEINBACH, H. B., Professor of Zoology, University of Minnesota.
STEWART, DOROTHY R., Associate Professor of Zoology and Physiology, Rockford College.
STRAUS, WILLIAM L., JR., Associate Professor of Anatomy, Johns Hopkins University.
SZENT-GYÖRGYI, A. E., Szent-Györgyi Research Foundation.
TAHMISIAN, THEODORE N., Associate Scientist, Argonne National Laboratories.
TAYLOR, WM. RANDOLPH, Professor of Botany, University of Michigan.
TEWINKEL, LOIS, Associate Professor of Zoology, Smith College.
TING, TE-PANG, Eli Lilly Fellow, Institute of Radiobiology, University of Chicago.
TRACY, H. C., Professor of Anatomy, University of Kansas.
TRINKAUS, J. PHILIP, Instructor in Zoology, Osborn Zoological Laboratory.

TUNG, TI-CHOW, Special Fellow, Rockefeller Foundation, Osborn Zoological Laboratory.
TYLER, ALBERT, Associate Professor of Embryology, California Institute of Technology.
TYLER, DAVID B., Research Associate, Carnegie Institute of Washington.
VILLEE, CLAUDE A., Associate in Biological Chemistry, Harvard Medical School.
WAINIO, WALTER W., Assistant Professor of Physiology, New York University College of Dentistry.
WALD, GEORGE, Professor of Biology, Harvard University.
WALKER, RUTH I., Professor of Botany, University of Wisconsin.
WARNER, ROBERT C., Assistant Professor of Chemistry, New York University College of Medicine.
WATERMAN, TALBOT H., Assistant Professor of Zoology, Yale University.
WHITE, ELIZABETH L., Instructor in Zoology, Washington University.
WHITING, ANNA R., Guest Investigator, University of Pennsylvania.
WHITING, P. W., Professor of Zoology, University of Pennsylvania.
WICHTERMAN, RALPH, Associate Professor, Temple University.
WOODWARD, ARTHUR A., JR., Instructor in Biology, Brown University.
WRINCH, DOROTHY, Lecturer in Physics, Smith College.
YUDKIN, WARREN H., Graduate Student, Yale University.
ZINN, DONALD J., Instructor in Zoology, Rhode Island State College.
ZORZOLI, ANITA, Instructor in Physiology, Washington University School of Dentistry.

Beginning Investigators, 1948

ANAGNOSTIS, IRENE P., Student, New York University.
ALSCHER, RUTH P., Instructor in Biology, Manhattanville College.
BOYLE, E. MARIE, Science Teacher, Baldwin School.
BULLOCH, JANE ANN, Student, University of Oklahoma.
COHEN, ARTHUR I., Student, University of Minnesota.
COOPERSTEIN, SHERWIN J., Student, New York University College of Dentistry.
CORET, IRVING A., Research Fellow, University of Pennsylvania.
EICHEL, HERBERT J., Graduate Student, New York University.
ESSNER, EDWARD S., Graduate Student, University of Pennsylvania.
FITCH, NAOMI, Assistant in Zoology, Columbia University.
GAGNON, ANDRE, Research Assistant, University of Pennsylvania.
GASVODA, BETTY M., Junior Scientist, Argonne National Laboratories.
GOODKIND, M. JAY, Student, Princeton University.
GOREAU, THOMAS F., Medical Student, University of Pennsylvania.
GREEN, JAMES W., Research Associate, Princeton University.
GREGG, JAMES H., Graduate Student, Princeton University.
HARDING, CLIFFORD V., JR., Graduate Student, University of Pennsylvania.
HAY, ELIZABETH D., Student, Smith College.
HIRSCHHORN, HENRY A., Student, New York University College of Dentistry.
HODGSON, EDWARD S., JR., Junior Instructor, Johns Hopkins University.
HOFFMAN, JOSEPH F., Student, University of Oklahoma.
HONEGGER, C. M., Instructor, Temple University.
HOPKINS, AMOS L., JR., Graduate Student, University of Pennsylvania.
JACOBSON, JULIUS H., Graduate Student, University of Pennsylvania.
JONES, GWEN MAXINE, Teaching Assistant, Northwestern University.
KELLY, JOHN W., Graduate Student, University of Pennsylvania.
KOZAM, GEORGE, Instructor, New York University College of Dentistry.
MARTIN, BARBARA ADELE, Assistant in Zoology, Barnard College.
MOSKOVIC, SAMUEL, Teaching Fellow, Graduate School, New York University.
NARDONE, ROLAND M., Laboratory Assistant, Fordham University.
NELSON, THOMAS CLIFFORD, Lecturer in Biophysics, Columbia University.
PADYKULA, HELEN A., Instructor, Wellesley College.
RAY, DAVID T., Graduate Student, University of Pennsylvania.
RIESER, PETER, Assistant Instructor, University of Pennsylvania.
ROSENBAUM, JOAN, Student, Columbia University.
ROSENBLUTH, RAJA, Graduate Student, Columbia University.
ROSSETTI, FIAMMETTA, Student, University of Chicago.

ROTHBERG, HARVEY, JR., Undergraduate, Princeton University.
SEAMAN, GERALD R., Graduate Assistant in Biology, Fordham University.
SUSCA, LOUIS A., Instructor, Fordham University.
SZE, L. C., Research Assistant, Columbia University.
WILSON, WALTER L., Research Assistant, University of Pennsylvania.

Research Assistants, 1948

BAILY, NORMAN A., Research Scientist, Columbia University.
BENSON, ELEANORE, Research Assistant, University of Missouri.
BERMAN, JACK H., Fellow in Anatomy, Western Reserve University.
BLUMENTHAL, GERTRUDE, Research Associate, University of Missouri.
CLARK, GRACE, Laboratory Technician, Columbia University.
COOPER, OCTAVIA, Research Assistant, Harvard University Medical School.
CRANE, ROBERT K., Chemist, Eli Lilly and Company.
CURTIS, PAUL, Student, Bethany College.
EGGERS, ANNETTE, Acting Instructor, Stanford University.
FASS, GEROME S., Research Technician, Rockefeller Institute for Medical Research.
FOLEY, MARY T., Research Assistant, Yale University.
FUSCO, EDNA MARIE, Research Assistant, Yale University.
GARNIC, JUSTINE, Graduate, Carnegie Institute of Technology.
GORDON, MARCIA, Research Assistant, Harvard Medical School.
HENLEY, CATHERINE, Research Assistant, University of North Carolina.
HICKSON, ANNA KELTCH, Research Chemist, Eli Lilly and Company.
HIMMELFARB, SYLVIA, Research Assistant, University of Maryland Medical School.
HOWARD, ROBERT S., Graduate Assistant, University of Miami.
JACOB, MIRIAM, Technician, Rockefeller Institute.
KEENEY, BELLE C., Stanford University.
KIMBERLY, PAUL E., Associate Professor, Des Moines Still College.
KIRK, MARJORIE J., Student, Harvard Medical School.
LEFEVRE, MARIAN E., Graduate Student, University of Vermont.
LEMM, FRANCES J., Research Assistant, Western Reserve University.
LESSE, HENRY, Medical Student, Jefferson Medical College.
LITOVCHICK, MORTIMER, Research Technician, Rockefeller Institute.
LOVE, LOIS H., Instructor, University of Pennsylvania.
LOVE, WARNER E., Assistant Instructor, University of Pennsylvania.
LOWENS, MARY D., Research Assistant, Harvard Medical School.
METCALF, JOHN S., JR., Medical Student, University of Maryland School of Medicine.
MITCHELL, CONSTANCE, Instructor, University of Delaware.
NACH, LUCILLE, Research Assistant, Western Reserve University.
PASSANO, LEONARD M., III, Graduate Student, Yale University.
PODOLSKY, BETTY, Research Assistant, University of Chicago.
RASKIND, JOSEPHINE B., Bryn Mawr College.
RAWLEY, JUNE, Student, University of Oklahoma.
RICH, ALEXANDER, Research Assistant, Harvard Medical School.
ROTH, ALEXANDER, Research Assistant, University of Kansas.
ROTHENBERG, MORTIMER A., Research Assistant, Columbia University.
SANDEEN, MURIEL I., Teaching Assistant, Northwestern University.
SEAMAN, ARLENE R., Assistant, Cornell University.
SHEDD, DONALD H., Dartmouth College.
SLATTERY, LEO F., Research Assistant, University of Chicago.
SPIEGEL, LEONARD E., Graduate Assistant in Botany, Northwestern University.
VAN HOESEN, DRUSILLA, Research Associate, University of Pennsylvania.
VOLKMAN, ALVIN, Student, University of Buffalo.
WALTERS, C. PATRICIA, Chemist Assistant, Eli Lilly and Company.
WEBB, H. MARGUERITE, Teaching Assistant, Northwestern University.
WETSTONE, HOWARD J., Research Assistant, Smith College.
WILSON, MARIE, Teaching Assistant, Northwestern University.
WINBLAD, JAMES N., Research Assistant, University of Kansas Medical School.

Library Readers, 1948

ABELL, RICHARD G., Resident in Psychiatry, College of Physicians and Surgeons.
ADLER, FRANCIS H., Professor of Ophthalmology, University of Pennsylvania.
ADLERSBERG, DAVID, Adjunct Physician, Mt. Sinai Hospital.
ANDERSON, RUBERT S., Assistant Professor of Physiology, University of Maryland Medical School.
BARTLETT, JAMES H., Professor of Physics, University of Illinois.
BERMAN, EVELYN M., Teacher, Montreal Protestant.
BLAIR, JOHN H., Assistant Professor of Physiology, University of Massachusetts.
BOROFF, DANIEL A., Research Bacteriologist.
CURTIS, W. C., Dean and Professor of Zoology Emeritus, University of Missouri.
DISCHE, ZACHARIAS, Assistant Professor of Biochemistry, College of Physicians and Surgeons.
EHRLICH, MIRIAM, Graduate Student, Yale University.
FOLEY, JOSEPH B., Graduate Student, Yale University.
FREUND, JULES, Chief, Public Health Research Institute of the City of New York.
GATES, R. RUGGLES, Research Fellow in Biology, Harvard University.
GRANT, MADELEINE P., Member of Science Faculty, Sarah Lawrence College.
GUDERNATSCH, FREDERICK, Retired Visiting Professor, New York University.
GUREWICH, VLADIMIR, Assistant Visiting Physician, Bellevue Hospital.
HANKE, HARRIETT, Teaching Fellow in Biology, New York University.
HENSLEY, RUTH A., Graduate Assistant in Zoology, University of Missouri.
HESS, ECKHARD H., The University of Chicago.
HILL, RUTH F., Assistant Physicist, Sloan-Kettering Institute.
HUTCHINGS, LOIS M., Instructor, Drew University.
KAPLAN, ANN E., Graduate Assistant, Mt. Holyoke College.
KAUPE, WALTER, Research Assistant, Massachusetts Institute of Technology.
KAUZMANN, WALTER, Assistant Professor of Chemistry, Princeton University.
KEANE, JOHN FRANCIS, Fordham University.
KEEFFE, MARY M., Assistant Professor of Biology, College of St. Thomas.
KRASNOW, FRANCES, Head of Department of Research, The Guggenheim Dental Foundation.
LEIGHTON, JOSEPH, Massachusetts General Hospital.
LEIKIND, MORRIS C., Head of Biology and Medicine Unit, Library of Congress.
LISI, ALFRED G., Instructor, Department of Pharmacology, Jefferson Medical College.
LOEWI, OTTO, Research Professor, New York University College of Medicine.
MCDONALD, SISTER ELIZABETH SETON, Professor of Biology, Mt. St. Joseph College.
MARTIN, ARTHUR W., Associate Professor of Physiology, University of Washington.
MATHEWS, ALBERT P., Carnegie Professor of Biochemistry Emeritus, University of Chicago.
MEYERHOF, OTTO, Research Professor, University of Pennsylvania.
MILLER, ELIZABETH M., Technician, Rockefeller Institute.
SCHILLER, PAUL H., Research Associate, Yerkes Laboratories.
SCHUH, JOSEPH E., Graduate Student, Columbia University.
SHAPIRO, HERBERT, Physiologist, National Institute of Health.
SHWARTZMAN, GREGORY, Head of Department of Bacteriology, Mt. Sinai Hospital.
SMITH, SYDNEY, Research Associate, University of Rochester.
STAUFFER, ROBERT C., Assistant Professor of History of Science, University of Wisconsin.
STERN, KURT G., Adjunct Professor of Biochemistry, Polytechnic Institute.
SULKIN, S. EDWARD, Professor and Chairman, Southwestern Medical College.
TAGNON, HENRY J., Associate Member, Sloan-Kettering Institute.
THERMAN, OLAF, Director of Laboratories, Pennsylvania Hospital.
TUTELMAN, HARRIET, Graduate Student, Johns Hopkins University.
WILLIER, B. H., Professor of Zoology, Johns Hopkins University.
YNTEMA, CHESTER L., Associate Professor of Anatomy, Syracuse University Medical School.

Students, 1948

BOTANY

ABBOTT, ROBINSON S., Student, Bucknell University.
BATTLEY, EDWIN H., Student, Harvard College.

BERNATOWICZ, ALBERT J., Student, Clark University.
COWEN, NAOMI, Student, Cornell University.
DIAMOND, RUDOLPH, Student, Syracuse University.
DOE, BARBARA, Instructor in Biology, Loretto Heights College.
EISENMAN, GEORGE, Student, Harvard University.
HANDLER, HOPE S., Student, Smith College.
HORWITZ, LEONARD, Student, College of the City of New York.
LAY, KO KO, Student, Washington University.
PRODELL, RITA C., Student, Drew University.
RUMELY, JOHN H., Student, Oberlin College.
SPRINGER, HELEN V., Student, Vassar College.
UMANZIO, DR. CARL B., Professor of Bacteria and Public Health, Kirksville College of Osteopathy.
WILLIAMS, LOUIS G., Associate Professor of Biology, Furman University.

EMBRYOLOGY

ANAGNOSTIS, IRENE P., Student, New York University.
CLARK, EUGENIE, Research Assistant, American Museum of Natural History.
CLARK, EDWARD C., Student, University of Massachusetts.
DANES, BETTY, Student, Mount Holyoke College.
DANIELS, EDWARD W., Research Assistant, University of Illinois.
EASTERLING, GEORGE R., Assistant Professor of Biology, Kent State University.
HAFFNER, RUDOLPH E., Student, Yale University.
HEALY, EUGENE A., Student, Columbia University.
HEATH, H. DUANE, Student, University of Chicago.
HODGSON, EDWARD S., JR., Junior Instructor, Johns Hopkins University.
JAFFEE, OSCAR C., Student, New York University.
JASKOSKI, BENEDICT J., Student, University of Minnesota.
JONES, EDWARD E., Student, University of North Carolina.
KENT, JOHN F., Assistant in Zoology, Cornell University.
MOULTON, JAMES M., Graduate Assistant in Biology, Williams College.
NACE, GEORGE W., Graduate Student, University of California, Los Angeles.
NADEAU, REV. LOUIS V., Biology Department, Fenwick High School.
OPPERMAN, JEAN ANN, Student, Seton Hill College.
PARKS, HAROLD F., Teaching Assistant, Cornell University.
RAECKE, MARJORIE J., Graduate Student, University of Nebraska.
RAUCH, HAROLD, Graduate Assistant, Brown University.
REICH, EDWARD, Undergraduate Assistant, McGill University.
RHODES, STANLEY A., Graduate Assistant, Duke University.
ROSSETTI, FIAMMETA, Student, University of Chicago.
ROTHBERG, HARVEY D., JR., Undergraduate, Princeton University.
SCHREIMAN, EVELYN S., Assistant Instructor, Rutgers University.
TODD, DORIS J., Student, Smith College.
WASHINGTON, DOROTHY A., Student, George Washington University.
WATSON, RUBY J., Student, Wheaton College.

PHYSIOLOGY

BAUER, M. H., Graduate Student, Princeton University.
BERNSTEIN, MAURICE H., Graduate Assistant, Washington University.
BONÉ, GEORGE J., Lecturer, Institute of Tropical Medicine, Antwerp, Belgium.
BRIGHAM, ELIZABETH H., Student, Rockefeller Institute for Medical Research.
CHENG, SZE-CHUH, Graduate Student, Brown University.
DIERMEIER, HAROLD F., Graduate Assistant, Syracuse University.
FLAGLER, ELIZABETH A., Student, Mount Holyoke College.
GREEN, FRANCES, Teaching Fellow, New York University.
HEROUX, OLIVIER, Graduate Student, Laval University, Quebec.
HOFFMAN, JOSEPH F., Student, University of Oklahoma.

JACOBSON, JULIUS H., II, Graduate Student, University of Pennsylvania.
JENCKS, WILLIAM P., Student, Harvard Medical School.
JOHNSON, AUDREY C., Graduate Student, Brooklyn College.
JUNQUEIRA, LUIZ C. U., Associate Professor, University of São Paulo, Brazil.
KIRSCHNER, LEONARD B., Research Assistant in Physiology, University of Wisconsin.
KUFF, DR. EDWARD L., Instructor, Washington University Medical School.
LESSLER, MILTON A., Teaching Fellow, New York University.
METZ, BERNARD, Graduate Assistant, Fordham University.
PALAY, SANFORD L., Visiting Investigator, Rockefeller Institute for Medical Research.
PARDEE, ARTHUR BECK, Post-Doctoral Fellow, University of Wisconsin.
PEASE, EVELYN A., Teaching Fellow, University of Michigan.
RANKIN, EUGENE M., Graduate Student, Tufts College.
SUTRO, PETER J., Graduate Student, Harvard University.
TARR, ELIZABETH H., Student, Stanford University.
ZEIDMAN, IRVING, Instructor, University of Pennsylvania School of Medicine.

ZOOLOGY

ALDRICH, FREDERICK A., Undergraduate Student Assistant, Drew University.
ARMSTRONG, RUTH A., Student, Vassar College.
BAIR, THOMAS D., Assistant, University of Illinois.
BARBER, DONALD S., Graduate Student, Amherst College.
BARNETT, ROBERT CHARLES, Student, University of Chicago.
BARRETT, JAMES M., Graduate Assistant in Zoology, Marquette University.
BELLINGER, PETER F., Student, Yale University.
BERGER, RUTH, Undergraduate Student, Goucher College.
BOHRN, MARIE T., Instructor in Geology, Mount Holyoke College.
BOUCOT, ARTHUR J., Student, Harvard University.
BROWN, HARLEY P., Assistant Professor, University of Oklahoma.
BURCH, CHARLES, Graduate Assistant, Cornell University.
COADY, MARTHA B., Student, Simmons College.
CORLISS, JOHN O., Graduate Assistant, New York University.
DEARDORFF, ALICE A., Graduate Assistant, Wesleyan University.
ECKL, BETTY A., Student, Mount Holyoke College.
ETZ, MONICA ZELDA, Undergraduate Student, Goucher College.
EWING, MARY J., Student, Pennsylvania College for Women.
FARROW, AUDREY P., Student, Wheaton College.
FERGUSON, ANNE V. D., Student, Elmira College.
GODSHALK, ELIZABETH L., Student, University of California, Los Angeles.
GUYSELMAN, JOHN B., Graduate Assistant, Northwestern University.
HENSLEY, RUTH A., Graduate Assistant in Zoology, University of Missouri.
HOLLAND, ROBERT A., Student, Drury College.
HOLZ, GEORGE G., JR., Graduate Assistant in Biology, New York University.
HUNTINGTON, CHARLES E., Student, Yale University.
ICHIKAWA, HIROKO, Student, Wilson College.
JENNER, CHARLES E., Graduate Student, Harvard University.
KEEVIL, CHARLES S., JR., Graduate Assistant, Amherst College.
KETTERER, JOHN J., Graduate Assistant in Biology, New York University.
KUTA, VIRGINIA A., Student, DePaul University.
LAMBERT, FRANCIS L., Student Assistant in Zoology, George Washington University.
LEWIS, FRANKLIN B., Student, Union College.
LOUD, ALDEN V., Student, Massachusetts Institute of Technology.
MCKIBBEN, JULIET N., Instructor, Carnegie Institute of Technology.
MIRSKY, REBA, Student, Indiana University.
NERAD, JOSEPHINE, Graduate Student, DePaul University.
NESE, ROSE M., 409 Western Avenue, East Pittsburgh, Pennsylvania.
O'MALLEY, BENEDICT B., Instructor in Botany and Anatomy, Fordham University.
ORSKI, BARBARA, Student, Hunter College.
PELOQUIN, STANLEY J., Student, Marquette University.

PERRY, THOMAS O., Student, Harvard University.
PROVASOLI, LUIGI, Research Associate, Haskins Laboratories.
REICHART, RUTH, Student, Radcliffe College.
ROGERS, KAY T., Teaching Fellow, Harvard University.
SACHS, BARBARA C., Student, Oberlin College.
SHOMAY, DAVID, Student, Long Island University.
SLOBODKIN, LAWRENCE B., Graduate Student, Yale University.
STEVENS, ARTHUR L., Research Associate, University of Notre Dame.
THOMAS, LYELL J., JR., Student, Oberlin College.
UREY, GERTRUDE E., Student, Swarthmore College.
WILLCOX, BARBARA L., Student, Oberlin College.
WILSON, CAROLYN E., Graduate Assistant, State University of Iowa.
WUNDER, CHARLES C., Student, Washington and Jefferson College.

3. THE LALOR FELLOWS

Senior Fellow: ERNEST BALDWIN, University of Cambridge, England.
Junior Fellows: AVRAM GOLDSTEIN, Harvard Medical School.
JOSEPH LEIN, Syracuse University.
HAROLD PERSKY, Michael Reese Hospital, Chicago.
WARREN YUDKIN, Yale Medical School.
Reappointments: IRVING KLOTZ, Northwestern University.
ARNOLD LAZAROW, Western Reserve.
BENJAMIN LIBET, University of Chicago.
CLAUDE VILLEE, Harvard Medical School.

4. THE ATOMIC ENERGY COMMISSION FELLOWS

RICHARD ABRAMS, Institute of Radiobiology, University of Chicago.
D. E. COPELAND, Brown University.
T. P. TING, University of Chicago.
J. J. WOLKEN, University of Pittsburgh.
A. A. WOODWARD, Brown University.

5. TABULAR VIEW OF ATTENDANCE

	1944	*1945*	*1946*	*1947*	*1948*
INVESTIGATORS—Total	193	212	267	299	326
Independent	112	138	175	182	183
Under instruction	11	10	29	36	42
Library readers	50	38	38	36	50
Research assistants	20	26	25	45	51
STUDENTS—Total	75	96	122	131	123
Zoology	37	55	57	55	54
Embryology	23	23	30	33	29
Physiology	10	13	26	26	25
Botany	5	5	9	17	15
TOTAL ATTENDANCE	276	308	389	430	449
Less persons registered as both students and investigators	1			2	6
	275				443
INSTITUTIONS REPRESENTED—Total	106	124	141	148	158
By investigators	74	100	102	114	117
By students	41	49	56	56	68
SCHOOLS AND ACADEMIES REPRESENTED					
By investigators	1	2	2	1	3
By students	2	2		1	
FOREIGN INSTITUTIONS REPRESENTED					
By investigators	2	1	7	7	8
By students	3		5	3	4

6. Subscribing and Cooperating Institutions, 1948

Cooperating Institutions

Amherst College
Boston Dispensary
Brooklyn College
Brown University
Bryn Mawr College
Carnegie Inst. of Washington
Catholic University of America
College of Mt. St. Joseph-on-the-Ohio
College of Physicians and Surgeons
Columbia University
Cornell University
Duke University
Fordham University
Goucher College
Harvard University
Harvard University Medical School
Johns Hopkins University
Johns Hopkins University Medical School
Johnson Foundation
Eli Lilly Company
Madison Foundation
Massachusetts Inst. of Technology
Miami University
Mount Holyoke College
Mount Sinai Hospital
New York University College of Medicine
New York University School of Dentistry
New York University Washington Square College
Northwestern University
Oberlin College
Pennsylvania College for Women
Princeton University
Rockefeller Foundation
Rockefeller Institute for Medical Research
Seton Hill College
Sloan-Kettering Institute
Smith College
State University of Iowa
Syracuse University Medical School
Temple University
Tufts College Medical School
Union College
University of Chicago
University of Illinois
University of Kansas
University of Maryland School of Medicine
University of Michigan
University of Minnesota
University of Missouri
University of Pennsylvania
University of Pennsylvania Medical School
University of Rochester
University of Vermont Medical School
University of Virginia
Vassar College
Washington University
Washington Univ. School of Dentistry
Wesleyan University
Western Reserve University
Woods Hole Oceanographic Institution
Yale University

Subscribing Institutions

Argonne National Laboratory
Belgium American Educational Fund
California Institute of Technology
College of Charleston
College of the City of New York
DesMoines Still College of Osteopathy
Drury College
Elmira College
Georgetown University School of Medicine
Hunter College
Institute for Cancer Research
Institute for Muscle Research
Institute of Pennsylvania Hospital
W. K. Kellogg Foundation
Marquette University
National Cancer Institute
National Institute of Health
North Carolina State College of Agriculture & Engineering
Public Health Research Institute of New York City
Radcliffe College
Southwestern Medical College
University of Delaware
University of Massachusetts
University of Oklahoma
University of Pittsburgh
Wheaton College

7. The Friday Evening Lectures, 1948

Friday, July 2
Dr. William R. Duryee ..... "The Structure and Function of Egg Nuclei."
Friday, July 9
Dr. J. T. Bonner ..... "Morphogenetic Movements in the Amoeboid Slime Molds."

Friday, July 16
Dr. Ernest Baldwin ..... "Nemathelminthes and Antihelminthics."
Friday, July 23
Dr. Paul C. Aebersold ..... "Recent Developments in the Use of Isotopes in Biology and Medicine."
Friday, July 30
Dr. A. H. Sturtevant ..... "Closely Similar Genes at Identical Loci."
Friday, August 6
Dr. John O. Hutchens ..... "Biological and Chemical Activities of Nitrogen Mustards."
Friday, August 13
Prof. E. W. Sinnott ..... "The Problem of Size Determination in Plants."
Friday, August 20
Dr. Paul Weiss ..... "Nerve Growth and the Problem of Synthesis of Protoplasm."
Friday, August 27
Prof. Karl F. Bonhoeffer ..... "The Mechanism of Chemical Rhythmical Reaction."

Other Lectures

Wednesday, August 4
Dr. L. V. Foster ..... "Advance in Optical Instrumentation."
Thursday, August 12
Dr. George Lower ..... "Kodachromes of Marine Life at Woods Hole and Bermuda."
Wednesday, August 18
Dr. P. S. Galtsoff ..... "Biological Explorations in the Gulf of Panama."

8. Seminars, 1948

July 6
Arnold Lazarow ..... "Sulfhydral Metabolism of the Beta Cell: Its Relation to the Development of Diabetes."
Shlomo Hestrin ..... "Action Pattern of Crystalline Muscle Phosphorylase."
Rudolph Keller ..... "Vital Staining in Combined Ultra Violet and Daylight."
July 13
Milton Levy and Evelyn Slobodiansky "The Arrangement of Amino Acids in Silk —an Application of Isotopic Derivative Analysis."
Anita Zorzoli ..... "The Histochemical Localization of Alkaline Phosphatase in Mouse Bones of Different Ages."
B. Eichel, S. Cooperstein and W. W. Wainio ..... "A Partial Separation of the Cytochromes."
July 20
K. B. Augustinsson ..... "On the Specificity of Cholinesterase."
David Nachmansohn ..... "Effect of Anticholinesterases in conduction."
M. A. Rothenberg ..... "Rate of Electrolyte Penetration into the Nerve Interior."

July 27

F. W. Whiting and Bertina M. Blauch ..... "Reproductive Economy and the Genetic Block to Free Oviposition in the Chalcidoid Wasp, Melittobia."

Luiz C. V. Junqueira ..... "Biochemical and Histochemical Observation on the Sexual Dimorphism of Mice Submaxillary Glands."

Alfred E. Bliss ..... "Extraction of Purified Squid 'Visual Purple.'"

James A. Miller ..... "PH Estimations in Reconstituting Tubularia Stems."

August 3

Ivor Cornman ..... "Inhibition of Sea Urchin Egg Cleavage by a Series of Substituted Carbamates."

Alfred Marshak ..... "A Nuclear Precursor to Ribo- and Desoxyribonucleic Acids."

A. M. Shanes ..... "The Effects of Excitatory and Blocking Mechanism Supporting the Resting Potential of Nerve."

August 10

James W. Green ..... "The Relative Rate of Penetration of the Lower Fatty Acids into Beef Red Cells."

F. R. Hunter ..... "Osmotic Hemolysis in Hypertonic Solutions."

Harold Persky ..... "Hippuric Acid Excretion in Anxiety States."

Dorothy Wrinch ..... "Biological Specificity."

August 17

R. K. Crane, E. G. Ball, and A. K. Solomon ..... "Incorporation of Carbon Dioxide into Organic Linkage by Retina."

Claude A. Villee, M. Lowens, M. Gordon, E. Leonard, and A. Rich ..... "The Synthesis of Nucleoproteins in Developing Arbacia Studied with the Aid of P32."

Avram Goldstein ..... "Mechanisms of Interaction of Inhibitors with Plasma Cholinesterase."

E. DeRobertis ..... "Ultrastructure of the Nerve Axon."

9. Members of the Corporation, 1948

1. Life Members

Beckwith, Dr. Cora J., Vassar College, Poughkeepsie, New York.
Billings, Mr. R. C., 66 Franklin Street, Boston, Massachusetts.
Calvert, Dr. Philip P., University of Pennsylvania, Philadelphia, Pennsylvania.
Cole, Dr. Leon J., College of Agriculture, Madison, Wisconsin.
Conklin, Prof. Edwin G., Princeton University, Princeton, New Jersey.
Cowdry, Dr. E. V., Washington University, St. Louis, Missouri.
Jackson, Mr. Chas. C., 24 Congress Street, Boston, Massachusetts.
Jackson, Miss M. C., 88 Marlboro Street, Boston, Massachusetts.
King, Mr. Chas. A.
Kingsbury, Prof. B. F., Cornell University, Ithaca, New York.

LEWIS, PROF. W. H., Johns Hopkins University, Baltimore, Maryland.
MEANS, DR. J. H., 15 Chestnut Street, Boston, Massachusetts.
MOORE, DR. GEORGE T., Missouri Botanical Gardens, St. Louis, Missouri.
MOORE, DR. J. PERCY, University of Pennsylvania, Philadelphia, Pa.
MORGAN, MRS. T. H., Pasadena, California.
NOYES, MISS EVA J.
PORTER, DR. H. C., University of Pennsylvania, Philadelphia, Pennsylvania.
SCOTT, DR. ERNEST L., Columbia University, New York City, New York.
SEARS, DR. HENRY F., 86 Beacon Street, Boston, Massachusetts.
SHEDD, MR. E. A.
STRONG, DR. O. S., Columbia University, New York City, New York.
WAITE, PROF. F. C., 144 Locust Street, Dover, New Hampshire.
WALLACE, LOUISE B., 359 Lytton Avenue, Palo Alto, California.

2. REGULAR MEMBERS

ADAMS, DR. A. ELIZABETH, Mount Holyoke College, South Hadley, Massachusetts.
ADDISON, DR. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.
ADOLPH, DR. EDWARD F., University of Rochester Medical School, Rochester, New York.
ALBAUM, DR. HARRY G., Biology Dept., Brooklyn College, Brooklyn, N. Y.
ALBERT, DR. ALEXANDER, Mayo Clinic, Rochester, Minnesota.
ALLEE, DR. W. C., The University of Chicago, Chicago, Illinois.
AMBERSON, DR. WILLIAM R., Department of Physiology, University of Maryland, School of Medicine, Baltimore, Md.
ANDERSON, DR. RUBERT S., Department of Physiology, University of South Dakota, Vermillion, South Dakota.
ANDERSON, DR. T. F., University of Pennsylvania, Philadelphia, Pennsylvania.
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STUDIES ON THE MECHANISM OF ACTION OF IONIZING RADIATIONS. IV. EFFECT OF X-RAY IRRADIATION ON THE RESPIRATION OF SEA URCHIN SPERM

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It has been maintained by a number of investigators (see reviews by Scott, 1937; Fricke, 1934; Packard, 1931) that the respiration of single cells is strikingly resistant to the action of ionizing radiations, and this belief has been the basis for ignoring the role of enzyme inhibition when explaining the mechanism of action of ionizing radiations on living cells. In fact, Chesley (1934) reported that the respiration of sea urchin eggs, both fertilized and unfertilized, was unaffected by x-ray irradiation with as much as 43,000 r. Henshaw (1932, 1940) found delay in cleavage whether the eggs or the sperm were irradiated, but the smallest amount of irradiation used in these experiments was 4000 r. Evans et al. (1942) confirmed Henshaw's work on sea urchin sperm and reported that inhibition of fertilization by x-rays could be partially prevented by the addition of certain organic substances, as had previously been found by Dale (1940) when he discovered this protective effect against enzyme inhibition by x-rays. In previous reports it has been shown that the respiration of tissue slices from rats irradiated with doses of x-rays below 500 r was definitely inhibited (Barron, 1947) and that the respiration of grasshopper eggs was inhibited with doses of x-rays as low as 10 r (Tahmisian and Barron, 1946). It was considered important, in view of these last experiments, to reinvestigate the problem of the respiration of irradiated single cells. We present in this paper data on the effect of x-rays on the respiration of sea urchin (*Arbacia punctulata*) sperm.

Experimental

Sperm was obtained by cutting circularly the soft tissues of the sea urchin. By this process sperm was shed in small syracuse dishes. Sperm from several urchins was collected in graduated centrifuge tubes after filtration through gauze. Filtered sea water was added to fill up the centrifuge tube. Sperm was then separated by short centrifugation (10 minutes at 2000 r.p.m.), the supernatant fluid was discarded, and the remaining sperm was brought to the desired dilution starting with a stock dilution of 1 : 10 or 1 : 20. The stock suspension was thoroughly shaken and an aliquot was taken for dry weight. To obtain the dry weights, 0.5 cc. of this suspension was added to specially hardened pyrex tubes and was centrifuged in a Beams type air-driven high speed centrifuge with 85 lbs. air pressure for 10 minutes. The fluid was withdrawn and the tubes were dried overnight at 110°. The sperm dilution chosen for the irradiation experiments was 1 : 200. This sperm suspension (1.2 cc.) was pipetted into small glass vials of 15 mm. diameter and 20 mm. height.

These vials (20 for each set of experiments) were placed in an aluminum holder resting in a glass container full of cracked ice. X-ray irradiation was performed by Mr. Hyde of the Department of Radiology of the Marine Biological Laboratory. The x-ray machine operated at 182 Kv peak voltage and a current of 25 ma. through each tube. A filter of 0.2 mm. Cu was used. The measurement of respiration started 40 minutes after irradiation.

Effect of dilution on the respiration of sea urchin sperm

Gray found in 1928 that the respiration of sea urchin sperm increased on dilution, but no quantitative study of this phenomenon has yet been reported. Although Hayashi (1946) attempted to measure this dilution effect, his techniques of measurement of O_2 uptake and of dilution were faulty, and his paper gives no data but rough figures. It was, therefore, necessary to determine the optimum sperm dilution which would give the maximum Q_{O_2} values (c.mm. of O_2 uptake per mg. dry weight per hour), and steady rates of respiration for at least one hour. A large number of experiments were performed for this purpose with different sperm dilutions, from 1:10 to 1:1000. The O_2 uptake increased steadily up to a dilution of 1:200. It started to decline when the dilution was increased to 1:400. A dilution of 1:1000

TABLE I

The effect of dilution on the respiration of sea urchin sperm (Arbacia punctulata). *Values, Q_{O_2}, give c.mm. O_2 uptake per mg. dry weight per hour*

Dilution	Q_{O_2}
1:10	1.7
1:30	3.6
1:100	10.0
1:150	14.6
1:200	19.6
1:400	17.5
1:1,000	2.0
1:1,600	None

gave an O_2 uptake as small as that of sperm at a dilution of 1:10; furthermore, the respiration almost ceased at the end of one hour. When the sperm was diluted to 1:1600, there was no measurable respiration (Table I). This lack of respiration was not due to lack of sensitivity of the Warburg manometric technique, for when measurements were made with the very sensitive Cartesian diver technique of Linderstrom-Lang as modified by Claff [1] similar negative results were obtained. The increase in respiration of sperm with dilution is undoubtedly due to greater motility in the dilute solutions.

When experiments were performed on one sample of pooled sperm, the values agreed within 10 per cent. The experiments performed on successive days with different sperm suspensions and the same dilution did not give reproducible values. The average Q_{O_2} value of 29 separate experiments performed on separate days (each experiment in triplicate) with a sperm dilution of 1:200 was 19.6 ± 3.9, i.e., with 20 per cent variation. Sperm dilutions of 1:200 could be kept at 3° for six hours with little decrease in respiration (11 to 14 per cent).

[1] Personal communication.

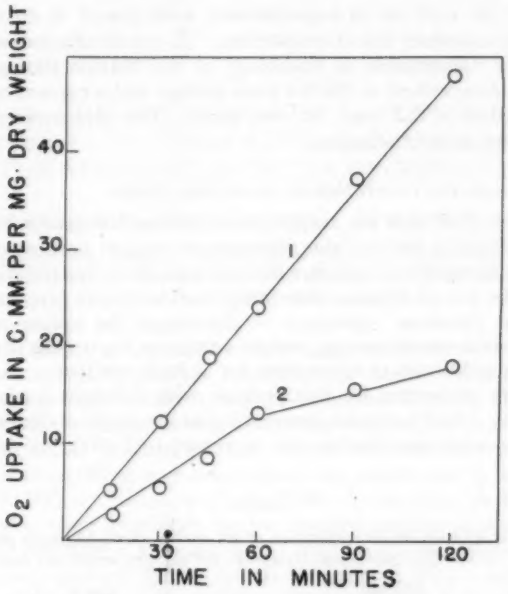

FIGURE 1. The effect of x-ray irradiation on the respiration of sea urchin sperm. Irradiation, 20,000 r. 1. Control; 2. Irradiated sperm.

It was not possible to obtain similar values in the O_2 uptake when the sperm was suspended in boiled sea water or in artificial sea water. The respiration of sperm in these cases was always much lower (20 to 40 per cent less).

Effect of x-rays on the respiration of sea urchin sperm

Once it was established that the optimum dilution for the measurement of sperm respiration was 1:200, all the experiments on the effect of x-ray irradiation were

TABLE II

Effect of x-rays on the respiration of sea urchin sperm. Dilution 1:200. Irradiation, 20,000 r. Figures give c.mm. O_2 uptake per mg. dry weight per hour

Exp. no.	Control	Irradiated	Inhibition (per cent)
1	14.8	6.0	59.4
2	16.2	3.8	76.6
3	12.0	5.0	58.4
4	23.9	8.9	62.8
5	18.3	5.3	71
6	13.1	2.8	79
7	29.6	17.6	54
8	18.6	10.6	43

performed with sperm suspensions so diluted. The sperm suspensions from 20 irradiated vials were collected in an Erlenmeyer flask from which 3 cc. were pipetted to each Warburg vessel. Thus, every experiment consisted of six control vessels and six vessels with irradiated sperm. Because of the reports in the literature on the resistance of respiration to x-ray irradiation, it was decided to start with 20,000 r. Such irradiation produced a marked inhibition of respiration, which increased in the second hour (Fig. 1). The degree of inhibition varied from sample to sample in all experiments. As an example of this variation the data of experiments of irradiation with 20,000 r are given in Table II. From this x-ray dose the amount of irradiation was diminished to 100 r with which an inhibition of 10 per cent was observed (Table III). The O_2 uptake inhibition on irradiation with 100 r did not increase in the second hour after irradiation (Fig. 2). In previous work

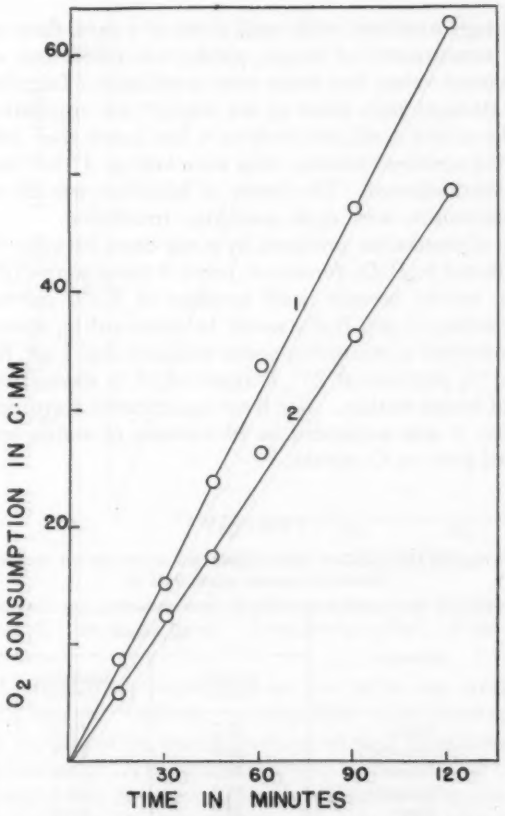

FIGURE 2. The effect of x-ray irradiation on the respiration of sea urchin sperm. Irradiation, 100 r. 1. Control; 2. Irradiated sperm.

TABLE III

The effect of x-rays on the respiration of sea urchin sperm. Sperm dilution 1:200. Q_{O_2} values, c.mm. O_2 uptake per mg. dry weight per hour

X-ray dose (r)	Q_{O_2} values		Inhibition (per cent)
	Control (c.mm.)	X-ray (c.mm.)	
20,000	22	7.5	66
10,000	19	13.3	30
1000	28	19.8	22
500	18	15.5	14
100	20	18	10

with grasshopper eggs irradiated with small doses of x-rays, there was inhibition of respiration when measurement of the O_2 uptake was made soon after irradiation, but a return to normal values five hours after irradiation (Tahmisian and Barron, 1947). Several attempts were made to see whether the respiration of sea urchin sperm inhibited by x-rays could also recover a few hours after irradiation. Soon after irradiation the sperm-containing vials were kept at 3° for five hours and the respiration was then measured. The degree of inhibition was the same as that obtained when measurements were made soon after irradiation.

The inhibition of respiration produced by x-ray doses between 1000 r and 100 r could not be attributed to H_2O_2 formation (even if there were H_2O_2 formation on irradiation of sea water) because small amounts of H_2O_2 increased respiration. Furthermore, a portion of this H_2O_2 would be destroyed by sperm catalase (Fig. 3). The catalase content of sea urchin sperm was such that 1 mg. dry weight would produce 33 c.mm. O_2 per hour at 25°, a figure which is eleven times less than the catalase content of mouse testicle. For these experiments sperm was washed three times in sea water, it was suspended in 10 volumes of water, and homogenized. Sperm thus treated gave no O_2 uptake.

TABLE IV

Effect of x-rays on the oxidation of succinate and acetate by sea urchin sperm. Substrate concentration, 0.01 M

X-ray dose (r)	Substrate	Q_{O_2} values		Inhibition (per cent)
		Control (c.mm.)	Irradiation (c.mm.)	
100	None	22.2	19.3	13
100	Succinate	30.0	22.8	24
100	None	20.5	18.0	12
100	Acetate	25.0	18.3	27
1000	None	20.5	15.0	26.9
1000	Succinate	27.2	20.0	38

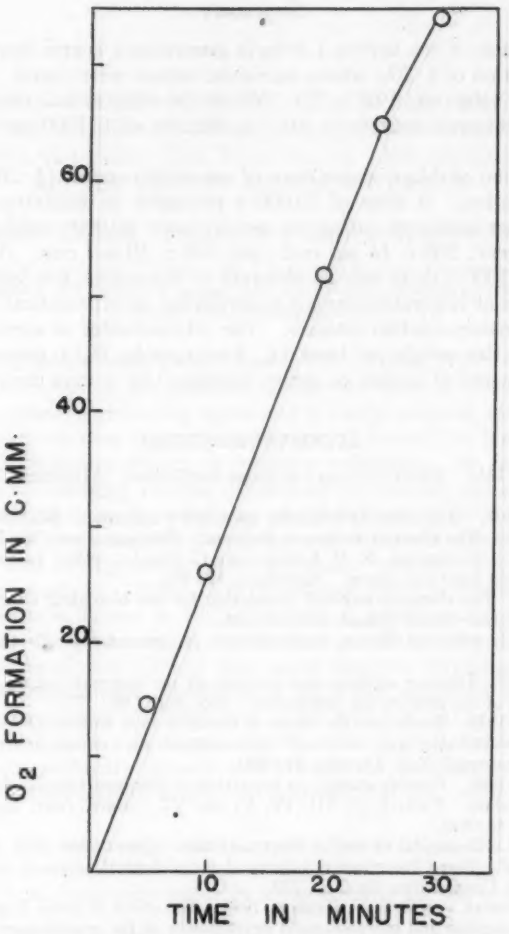

FIGURE 3. Catalase in sea urchin sperm. Buffer, phosphate, 0.01 M, pH 6.8; H_2O_2 0.01 M. Temperature 25°; Sperm, 0.1 cc. of 1: 10 suspension in H_2O, 1.8 mg. dry weight.

A number of intermediary metabolites are utilized by sea urchin sperm with an increase in the O_2 uptake. Of these, the utilization of succinate and of acetate (as measured by the increase of O_2 uptake) was tested after irradiation with 100 r. In both cases the inhibition of O_2 uptake was greater in the presence of substrates (Table IV), an indication that the enzymes for the oxidation of succinate and of acetate which belong to the group of sulfhydryl enzymes, are quite sensitive to the inhibiting effect of x-rays.

Summary

The respiration of sea urchin (*Arbacia punctulata*) sperm increased with dilution up to a dilution of 1:200, where maximum values were found. At this dilution the average Q_{O_2} value was 19.6 ± 3.9. When the dilution was increased to 1:1000 the respiration dropped sharply to 2.0. A dilution of 1:1600 gave no measurable respiration.

The respiration of dilute suspensions of sea urchin sperm (1:200) was inhibited by x-ray irradiation. A dose of 20,000 r produced an inhibition of 66 per cent which was further increased during the second hour; 10,000 r inhibited 30 per cent; 1000 r, 22 per cent; 500 r, 14 per cent; and 100 r, 10 per cent. When sperm was irradiated with 1000 r there was no recovery of respiration five hours after irradiation. Inhibition of respiration cannot be attributed to hypothetical H_2O_2 formation, for sperm suspensions contain catalase. The catalase value of sperm is 33 c.mm. O_2 formed by 1 mg. dry weight per hour, i.e., 3 micromoles H_2O_2 destroyed. On addition of succinate and of acetate to sperm irradiated by x-rays the O_2 uptake inhibition increased.

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STUDIES ON THE MECHANISM OF ACTION OF IONIZING RADIATIONS. V. THE EFFECT OF HYDROGEN PEROXIDE AND OF X-RAY IRRADIATED SEA WATER ON THE RESPIRATION OF SEA URCHIN SPERM AND EGGS

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It has been known for some time (Risse, 1929; Fricke, 1934) that when water is irradiated with x-rays in the presence of oxygen there is formation of H_2O_2. Since H_2O_2 is a powerful oxidizing agent and it easily oxidizes sulfhydryl groups, it was reasonable to assume that this substance, if formed on irradiation, would contribute to the biological effects of ionizing radiations. In fact, Barron and Dickman (1949) on studying enzyme inhibitions by ionizing radiations, and Barron and Flood [1] on studying the oxidation of 2,3-dithiopropane and of glutathione by x-rays, were able to distinguish the H_2O_2 contribution to this oxidation by the use of catalase. The French investigators Loiseleur, Latarjet, and Caillot (1941), and Loiseleur and Latarjet (1942) have postulated that the primary effect of irradiation in aqueous solutions is H_2O_2 formation, which would thus become of importance in the interpretation of the mechanism of ionizing radiations. The same view is held by Evans (1947) who found that the fertilizing power of sea urchin sperm is decreased when suspended in sea water irradiated with large doses of x-rays. Evans attributed this inhibition to the action of H_2O_2 seemingly formed on irradiation of sea water. In living cells the role of H_2O_2 becomes more complicated because the sulfhydryl groups which might be oxidized by this agent not only are present in the protein moiety of enzymes, but also exist as non-protein sulfhydryl groups.

We present in this paper experiments on the effect of H_2O_2 and of x-ray irradiated sea water on the respiration of sea urchin sperm. They do not support the belief that H_2O_2 is an important factor in x-ray toxicity on sea urchin sperm.

Experimental

Sea urchin sperm was obtained as described previously (Barron et al., 1949), and in all experiments a dilution of 1:200 was used. Freshly filtered sea water was irradiated at room temperature in large cellophane dishes and immediately after irradiation the sperm suspension was added, enough to make the desired dilution of 1:200.

The catalase added to irradiated sea water was prepared from beef liver according to Sumner and Dounce (1939). H_2O_2 was determined by the colorimetric method of Bonet-Maury (1944). An aliquot of the solution was taken (up to

[1] Unpublished experiments.

3 cc.) to which was added 0.5 cc. of 20 per cent H_2SO_4, 5 drops of the titanium sulfate reagent (10 g. $TiSO_4$ ground in mortar with 50 cc. H_2O and 20 g. H_2SO_4, D = 1.84, let stand 24 h., centrifuge, take the supernatant and add 20 g. H_2SO_4), and distilled water to 5 cc. The yellow color produced which is stable was read in a Beckman spectrophotometer at 4000 Å. With this method amounts of H_2O_2 from 0.5 micrograms to 25 micrograms could be determined. The respiration measurements were made at 25°.

Effect of H_2O_2 on the respiration of sea urchin sperm

Barron et al. (1948) have shown that sulfhydryl reagents when used in small concentrations increase the respiration of sea urchin sperm, whereas they inhibit it when the concentration is increased. To explain these opposite effects it was postulated that the cell contains two kinds of sulfhydryl groups: the non-protein sulf-

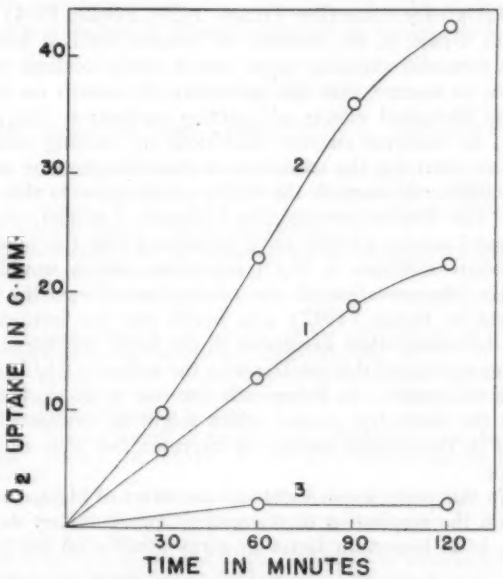

FIGURE 1. The effect of H_2O_2 on the respiration of sea urchin sperm. 1. Control; 2. H_2O_2, 1×10^{-5} M; 3. H_2O_2, 1×10^{-4} M.

hydryl groups (namely glutathione), which regulate the rate of respiration, and the sulfhydryl groups in respiratory enzymes. Destruction of the first would increase respiration, while destruction of the second would inhibit it. H_2O_2, an oxidizing agent of sulfhydryl groups, behaves in the same manner. At a concentration of 1×10^{-5} M it increased respiration, while 1×10^{-3} M inhibited it almost completely (Fig. 1). By altering the concentration of H_2O_2 between these two limits the effect on respiration is changed accordingly (Table I). In fertilized sea urchin eggs 1×10^{-4} M H_2O_2 increased the O_2 uptake 25 per cent (Fig. 2). If the effects of x-ray irradiation were mainly due to H_2O_2 formation, as postulated by

TABLE I

Effect of H_2O_2 on the respiration of sea urchin sperm. Sperm dilution, 1:200. Q_{O_2}, c.mm. O_2 uptake per mg. dry weight per hour

H_2O_2 Concentration (M)	Q_{O_2} values		Inhibition (−) or increase (+) (per cent)
	Control (c.mm.)	H_2O_2 (c.mm.)	
0.01	22.3	0	− complete
0.001	22.3	2.1	−90
0.0005	16.0	15.3	No effect
0.0001	20.2	33.7	+61.5
0.0001	22.0	42.0	+91

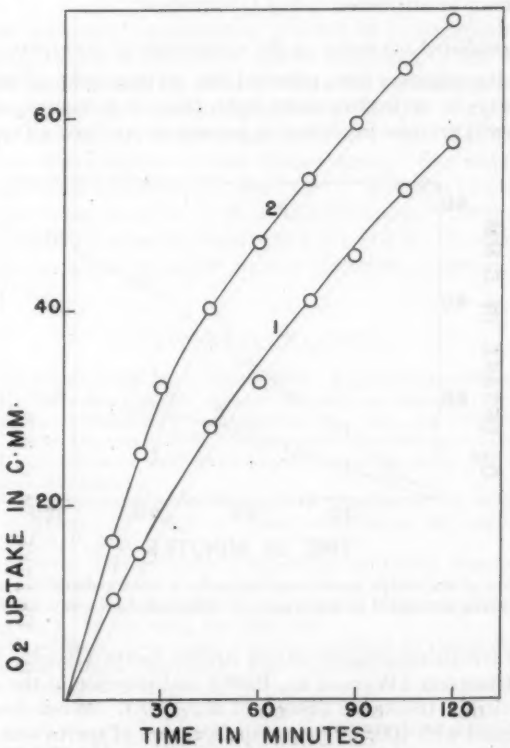

FIGURE 2. Effect of H_2O_2 on the respiration of fertilized sea urchin eggs. H_2O_2 concentration, 1×10^{-4} M; 1. Control; 2. H_2O_2.

TABLE II

Inhibition of sea urchin sperm respiration by x-ray irradiated sea water. X-ray dose, 100,000 r. Liver catalase (0.2 cc.) added immediately after irradiation; sperm suspension 5 minutes later

Experimental conditions	O_2 uptake		Inhibition (per cent)
	First hour (c.mm.)	Second hour (c.mm.)	
Control	29.7	53.5	
X-ray irradiated sea water	14.9	33.1	38
X-ray irradiation + catalase	16.2	36.6	34

Loiseleur et al. (1941, 1942) and by Evans (1947), x-ray irradiation at a dose of 1000 r would produce an increase in cell respiration.[2] Barron et al. (1949) have shown that on the contrary, respiration is inhibited. It must be concluded from these experiments that the effects of x-ray irradiation on the metabolism of sea urchin sperm cannot be attributed to H_2O_2 formation.

Effect of x-ray irradiated sea water on the respiration of sea urchin sperm

A number of investigators have reported that on irradiation of aqueous solutions, whether with x-rays or with ultra-violet light, there is formation of some unknown substance which will produce inhibition of growth of protozoa (Taylor et al., 1933),

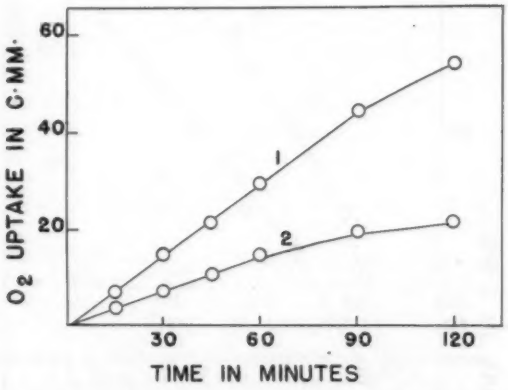

FIGURE 3. Inhibition of sea urchin sperm respiration by x-ray irradiated sea water. Irradiation, 200,000 r. 1. Sperm suspended in sea water; 2. Suspended in x-ray irradiated sea water.

inhibition of the fertilizing capacity of sea urchin sperm (Evans, 1947), inhibition of the growth of bacteria (Wyss et al., 1948), and increase in the mutation rate of *S. aureus* to penicillin resistance (Stone et al., 1947). When freshly filtered sea water was irradiated with 100,000 r, and a suspension of sperm was added to it soon

[2] From Bonet-Maury and Frilley's data (1944) it can be calculated that 1000 r would produce on irradiation of water about 1×10^{-5} M of H_2O_2.

after irradiation, there was an inhibition of respiration of 38 per cent. The inhibition was not affected by previous addition of catalase (Table II), an indication that the inhibition was not produced by H_2O_2. The inhibition increased when the dose of x-rays rose to 200,000 r (Fig. 3). Further evidence that this phenomenon was not produced by H_2O_2 was obtained by its detection with the titanous sulfate colorimetric method. While distilled water irradiated with 100,000 r gave 30 micrograms H_2O_2 per cc., filtered sea water irradiated with the same dose of x-rays gave no color at all. The lack of color formation was not due to the salt concentration of sea water, for on addition of H_2O_2 to sea water the color reaction appeared. We believe that the inhibition of respiration is due to the formation of stable organic peroxides formed on oxidation of the organic matter contained in sea water. It is quite possible that similar stable organic peroxides are formed on irradiation of biological fluids and that they contribute to the toxic effects of ionizing radiations. This problem is now under investigation.

Summary

Hydrogen peroxide at a concentration of 0.001 M produced almost complete inhibition of the respiration of sea urchin sperm suspended in sea water. At a concentration of 0.0005 M it had no effect. When the concentration was diminished to 0.0001 M it increased the respiration from 60 to 100 per cent.

When sperm was added to sea water irradiated with 200,000 r, there was a marked inhibition of respiration (about 60 per cent). Sea water irradiated with 50,000 r produced small inhibition (10 per cent). Addition of catalase previous to the addition of sperm had no effect at all on this inhibition. Furthermore, sea water irradiated with 200,000 r gave no positive test for H_2O_2. It is postulated that inhibition is due to the action of stable organic peroxides produced on irradiation of sea water.

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BACTERIA AND CELLULAR ACTIVITIES. IV. ACTION OF TOXINS ON RESPIRATION AND HEMOLYSIS OF DOGFISH ERYTHROCYTES AND ON RESPIRATION OF MARINE EGGS [1, 2]

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An attempt is being made in this laboratory to determine what effect bacterial toxins have on the functioning of cells. In the two preceding papers of this series (Hunter et al., 1949a, b) the action of seven toxins on the respiration and permeability of chicken erythrocytes was reported. In the present investigation the erythrocytes of the smooth dogfish, *Mustelus canis* and the unfertilized eggs of *Arbacia punctulata* and of *Asterias forbesei* were the cells studied.

Materials and Methods

The toxins were those previously used which were obtained locally and from the Lilly Laboratories and the Lederle Laboratories, to which the authors are indebted. Bacteriologically sterile techniques were used throughout except during the few minutes when hemolysis measurements were made. Tests for sterility were run at the end of each experiment as previously described (Hunter et al., 1949a). In addition, tests were also made for possible contamination by marine bacteria (see Waksman et al., 1933).

Oxygen consumption measurements were made using a Warburg apparatus at a temperature of 25° ± 0.1° C. Hemolysis times were measured using a photronic cell apparatus and a microammeter, since the more sensitive apparatus usually employed was not available. These measurements were made at room temperature which varied from day to day, but a water jacket surrounding the hemolysis chamber tended to minimize fluctuations during a series of readings. The time for hemolysis in 0.95 M ethylene glycol was measured in all cases.

Blood was procured under sterile conditions either by removal from the caudal vein with a hypodermic syringe, or by cutting the tail and allowing the blood to drain into a flask. Heparin was used as an anticoagulant in all experiments.

Immediately following the withdrawal of blood it was centrifuged at about 2000 r.p.m. for 10 minutes. The plasma and leucocytes were removed and the cells were carefully stirred. To 1 cc. of toxin was added 0.3 or 0.5 cc. of erythrocytes in each Warburg vessel which was immediately connected to its manometer and placed in the bath. Five to ten minutes were allowed for temperature equilibration, so that the initial readings were taken within 20 minutes or less of the time the toxins and cells were mixed. Controls were run using 1 cc. of sea water, 1 cc. of broth,

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[2] An abstract of a portion of this work appeared in the *Biol. Bull.*, **95**: 255, 1948.

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or 1 cc. of formalized toxins in place of the 1 cc. of toxins. In some experiments readings were made continuously, while in others the manometers were opened for about an hour following the first hour's readings. Cell counts and hematrocrit determinations were made as previously described.

Hemolysis measurements were made as follows: Equal volumes of blood and toxins were mixed and allowed to stand at room temperature. Controls were run substituting sea water, formalized toxins, formalized broth and formalized sea water for the toxins. From time to time 0.1 cc. aliquots were removed and added to 10 cc. of 0.95 M ethylene glycol in the hemolysis chamber. The course of hemolysis was followed by reading the microammeter at five-second intervals. The total exposure time to the toxins varied, but the maximum was 48 hours.

Formalized "controls" were used in both the respiratory and hemolysis experiments. These were the same stock suspensions previously described (Hunter et al., 1949a). As was true in the preceding work (Hunter et al., 1949b), these attenuated toxins were more satisfactory as "controls" for the permeability studies than for the respiratory studies. However, these attenuated toxins were more satisfactory as "controls" in the present respiration studies. Apparently the oxygen consumption of dogfish erythrocytes is not inhibited by formalin to such an extent as the oxygen consumption of chicken erythrocytes.

Unfertilized eggs of *Arbacia punctulata* and of *Asterias forbesei* were obtained under bacteriologically sterile conditions, using a technique similar to that described by Tyler et al. (1938). The dry weight of the eggs was obtained by centrifuging 1 cc. samples of the egg suspensions using an air turbine, removing the supernatant fluid and drying to constant weight.

In the majority of experiments in which eggs were used, 1 cc. of the toxin was placed in each Warburg vessel, 2 cc. of an egg suspension were added, the vessels were attached immediately to the manometers and five minutes were allowed for temperature equilibration. The first reading was taken within 20 minutes of the time the first vessel was prepared. Readings were taken at 10 minute intervals over a period of several hours.

Results

Erythrocyte respiration

The results of a typical experiment in which dogfish erythrocytes were exposed to the toxins are summarized in Table I. Respiration was also measured in the presence of formalized toxins (26 days after mixing formalin and toxin). In the case of the three toxins which accelerate respiration (*M. aureus, Cl. tetani* and *C. diphtheriae*), cells in the presence of the corresponding formalized toxins consumed oxygen at essentially the same rate as sea water controls. The other formalized toxins inhibited the rate of respiration but none more markedly than formalized sea water.

Hemolysis times

The effects of the toxins on hemolysis of dogfish erythrocytes in 0.95 M ethylene glycol are shown in Table II. It can be seen that the toxins of *Cl. perfringens* are most effective in altering the surface of the cells, for a half hour exposure is sufficient to bring about a marked increase in the rate of hemolysis. A 9 hour ex-

TABLE I

A typical experiment showing the rates of respiration of dogfish erythrocytes in the presence of various toxins

Toxin	μL O_2 per cc. of cells per hour		Remarks	
	1st hour	3–5 hours	1st hour	3–5 hours
Control	80	70	———	———
M. aureus	140	108	Marked acceleration	Marked acceleration—slightly less than 1st hour
Cl. tetani	160	85	Marked acceleration	Slight acceleration
C. diphtheriae	120	75	Marked acceleration	Little or no effect
Cl. septicum	80	70	No effect	No effect
Cl. perfringens	80	0	No effect	Complete inhibition (Possibly associated with hemolysis of the cells)
B. cereus	65	60	Slight inhibition	Slight inhibition
Str. pyogenes	50	<20	Moderate inhibition	Marked inhibition

posure to the toxins of *Str. pyogenes* or *B. cereus* or a 23 hour exposure to the toxins of *Cl. tetani* produce a less marked increase in the rate of hemolysis. The formalized tetanal toxins had the same effect as the toxins themselves, while the formalized streptococcal toxins, and to a lesser extent, the formalized cereus toxins, had an intermediate effect. These data might indicate either that the formalin had not completely inactivated the toxins responsible for the change in the surface of the cells, or that the changes in the cells are brought about by something other than the toxins. Although additional experiments would be required to demonstrate conclusively the explanation for these observations, there is little reason for believing that substances other than the toxins would be influencing the cells in this manner. The composition of the broth in this case is unknown. However, none of the broths studied in this laboratory has had any observable influence on the osmotic behavior

TABLE II

Hemolysis times for dogfish erythrocytes in 0.95 M ethylene glycol following exposure to various toxins

Time of exposure in hours	Organism producing toxin	Time in seconds for approximately 75 per cent hemolysis			
		Toxin	Control		
			Sea water	Formalized sea water	Formalized toxin
½	*Cl. perfringens*	11	31	31	29
9	*Str. pyogenes*	20	35	35	24
9	*B. cereus*	21	35	37	29
23	*Cl. tetani*	24	36	38	25
20	*Cl. septicum*	40	40	—	40
20	*C. diphtheriae*	30	35	25	30
20	*M. aureus*	35	35	25	30

of dogfish or other erythrocytes. In the case of tetanal toxin, the data obtained from the oxygen consumption studies suggest that the formalin does inactivate the toxin responsible for the change in respiration. As a tentative suggestion, therefore, one might assume that at least two tetanal toxins are present—one which accelerates respiration and is inactivated by formalin, and a second which alters the surface of the cell but which is not inactivated by formalin. It is hoped that future investigations will test the validity of this assumption.

The toxins of *Cl. septicum, C. diphtheriae,* and *M. aureus* have no effect on hemolysis following exposures of 20 hours.

Egg respiration

Figures 1 and 2 present the results of typical experiments using Arbacia and Asterias eggs respectively.

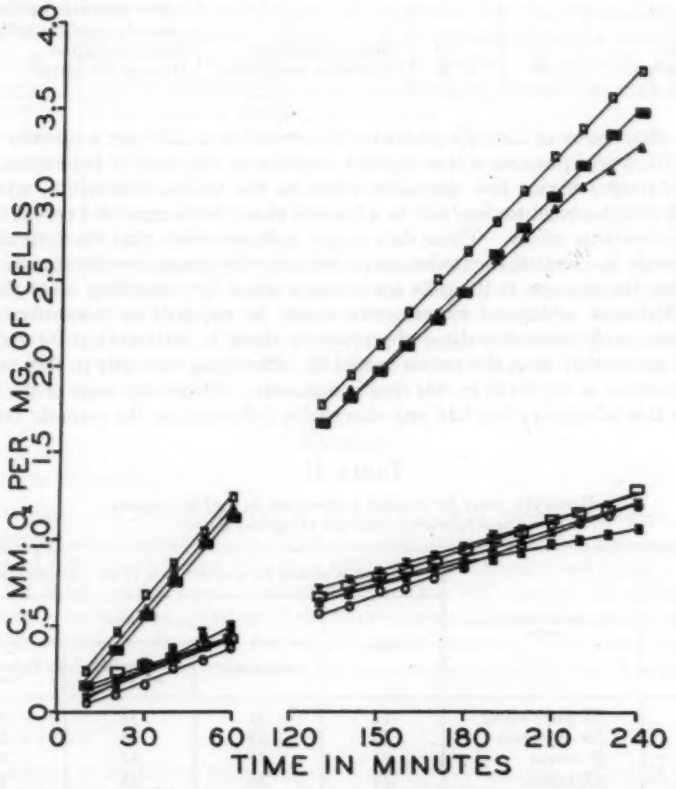

FIGURE 1. The effect of toxins on the oxygen consumption of unfertilized Arbacia eggs. Readings were taken for 60 minutes, then there was a 60 minute break and readings were again taken beginning at 120 minutes. □—*M. aureus;* ▬—*Cl. tetani;* △—*C. diphtheriae;* ○—Sea water; ▽—*Cl. septicum;* ▲—*B. cereus;* ■—*Str. pyogenes;* ▭—*Cl. perfringens.*

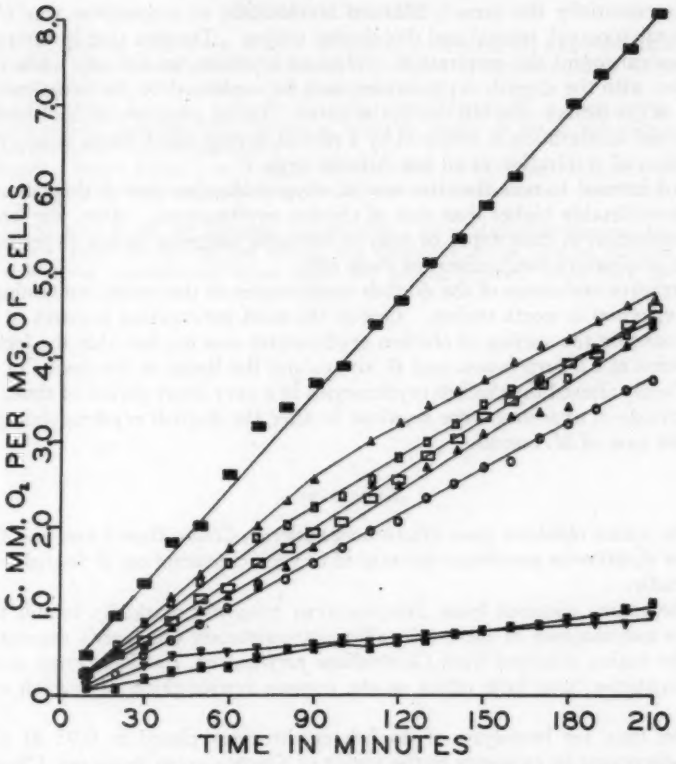

FIGURE 2. The effect of toxins on the oxygen consumption of unfertilized Asterias eggs. Readings were taken continuously for 210 minutes. ■—*Cl. tetani*; △—*C. diphtheriae*; □—*Cl. perfringens*; □—*M. aureus*; ▲—*B. cereus*; ○—Sea water; ■—*Str. pyogenes*; ▽—*Cl. septicum*.

These data are not conclusive since the heated toxins apparently had an effect similar to that of the toxins. However, it is interesting to note that the effects of these toxins on the eggs are in many respects the same as those on erythrocytes. In the case of Arbacia eggs, the toxins from *C. diphtheriae*, *Cl. tetani*, and *M. aureus* accelerate respiration. The significance of the lack of inhibition by the streptococcal toxins will be discussed in a future publication. In the case of Asterias eggs, the tetanal toxins clearly accelerate respiration; the diphtherial and staphylococcal toxins may accelerate slightly for the first hour or two; the perfringens toxins may accelerate slightly, while there is a suggestion of a delayed acceleration in the case of the toxins of *B. cereus*. The streptococcal toxins and those of *Cl. septicum* inhibit respiration.

DISCUSSION

A comparison between these data and those previously reported (Hunter et al., 1949a, b) shows that in general the action of the toxins on the four types of cells

studied is essentially the same. Marked acceleration of respiration was obtained only with micrococcal, tetanal and diphtherial toxins. The fact that in the presence of micrococcal toxins the respiration of chicken erythrocytes fell off, while this did not happen with the dogfish erythrocytes, may be explained by the hemolysis which occurred in the former case but not in the latter. In the presence of diphtherial toxins the initial acceleration is followed by a period during which there is no effect, or an inhibition of respiration of all but Arbacia eggs.

It is of interest to note that the rate of oxygen consumption of dogfish erythrocytes is considerably higher than that of chicken erythrocytes. Also, the sensitivity of the respiration of both types of cells to formalin suggests future experiments to study the respiratory mechanisms of these cells.

The relative resistance of the dogfish erythrocytes to the toxins containing lipid-splitting enzymes is worth noting. One of the most outstanding features of the action of toxins on the surface of chicken erythrocytes was the fact that the lecithinase in the toxins of *Cl. perfringens* and *B. cereus* and the lipase in the toxins of *M. aureus* markedly altered the chicken erythrocytes in a very short period of time. Much longer periods of exposure were required to alter the dogfish erythrocytes, particularly in the case of *M. aureus*.

Summary

1. The toxins obtained from *Micrococcus aureus, Clostridium tetani* and *Corynebacterium diphtheriae* accelerate the rate of oxygen consumption of dogfish erythrocytes initially.

2. The toxins obtained from *Streptococcus pyogenes* markedly inhibit the rate of oxygen consumption of these cells after approximately one hour's exposure.

3. The toxins obtained from *Clostridium perfringens, Bacillus cereus* and *Clostridium septicum* have little effect on the oxygen consumption of dogfish erythrocytes.

4. The time for hemolysis of dogfish erythrocytes placed in 0.95 M ethylene glycol is decreased by exposure to the toxins of *Streptococcus pyogenes, Clostridium perfringens* and *Bacillus cereus.*

5. There is a suggestion that the toxins of *Cl. tetani* have a similar effect, but formalized tetanal toxins also decrease hemolysis times.

6. The time for hemolysis of dogfish erythrocytes placed in 0.95 M ethylene glycol is not altered by the presence of the toxins of *Clostridium septicum, Corynebacterium diphtheriae* or *Micrococcus aureus.*

7. The toxins of *Clostridium tetani, Micrococcus aureus* and *Corynebacterium diphtheriae* increase the rate of oxygen consumption of both Arbacia and Asterias eggs.

8. The toxins of *Clostridium perfringens* increase the rate of oxygen consumption of Asterias eggs but have little effect on the respiration of Arbacia eggs.

9. The toxins of *Streptococcus pyogenes* decrease the rate of respiration of Asterias eggs but have little effect on Arbacia eggs.

10. The toxins of *Bacillus cereus* have little influence on the respiration of either Arbacia or Asterias eggs.

11. The toxins of *Clostridium septicum* inhibit the respiration of Asterias eggs but have little influence on Arbacia eggs.

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RESPIRATION AND WATER LOSS IN THE ADULT BLOWFLY, PHORMIA REGINA, AND THEIR RELATION TO THE PHYSIOLOGICAL ACTION OF DDT

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I. Introduction

The interrelations of respiration, muscular activity, water content and content of respirable and irrespirable solid material have been little investigated in insects, particularly at different metabolic rates. The discovery that DDT enhances weight loss and oxygen consumption (Laug, 1945; Ludwig, 1946; Läuger et al., 1946; Buck and Keister, 1946, 1947) makes this insecticide a possible tool for such studies. At the same time, opportunity is afforded for further investigation of the still obscure physiological action of DDT.

II. Materials and Methods

Unless otherwise noted, two- to four-day old adult males of the bluebottle fly, *Phormia regina* Meigen, were used. Larvae were grown on horse meat, and adults given only sucrose, water and fresh orange *ad libitum*. All experiments were done at 25° C. In handling flies, brief carbon dioxide narcosis was used (Williams, 1946). This also reduced feeding variations by causing regurgitation of crop contents (up to 8 per cent of live weight). Flies were poisoned by walking for 10 or 15 minutes in a 1-liter bottle containing 250 mg. DDT deposited as a thick, continuous, tenacious microcrystalline layer which formed slowly from minute supersaturated droplets left after the solvent (5 cc. anesthesia ether) was blown out by 2 minutes' exposure to a strong air current. All doses used were lethal.

For weight-loss experiments, the flies were narcotized, sexed, sorted into four equal groups of 25 to 50, and weighed in cylindrical wire cages, 1 × 2 inches. Two groups were then poisoned with DDT. One poisoned and one control group were set up over 10 per cent NaOH ("Wet Control" and "Wet DDT"), and the remaining two over NaOH pellets ("Dry Control" and "Dry DDT"), in the jar chambers illustrated in Figure 1. The vapor pressure of water was about 19 mm. of Hg over the solution and about 1 mm. over the pellets. Since carbon dioxide was completely absorbed in both types of chamber, conditions were similar to those in the Warburg flasks (see below). Set-up time was about 15 minutes for controls and 32 minutes for poisoned groups. Additional flies were killed with cyanide vapor at the start of some experiments and dried to constant weight at 55–60° C. for initial dry weight percentage.

All four groups were without food, and ostensibly without drinking water, during all experiments, and incoördination made ingestion impossible for the poisoned flies in any case. However, the Wet Control flies might have obtained some drink-

ing water if condensate appeared on the jar walls due to slight environmental temperature changes.

In the three types of weight loss experiments, flies were: (1) Removed briefly at intervals, weighed and replaced, thus giving the rate of gross weight loss. (2) Left for 10 hours, weighed, killed in cyanide vapor and dried to constant weight, giving the loss of dry weight (substrate) in 10 hours. (3) Left until dead, weighed, and dried to constant weight, giving the loss of solid matter previous to death.

Respiration was measured in 15–19 cc. Warburg flasks, one fly to a flask. High and low humidity were obtained by using, respectively, 0.33 cc. 8 per cent KOH, and solid KOH pellets as absorbents for carbon dioxide. The absorptive capacity of the moist surface of the pellets (240 mm^2) was at least as great as that of the 15 by 30 mm. ammonium-free filter paper fan in the "wet" flasks, and the risk of creep-

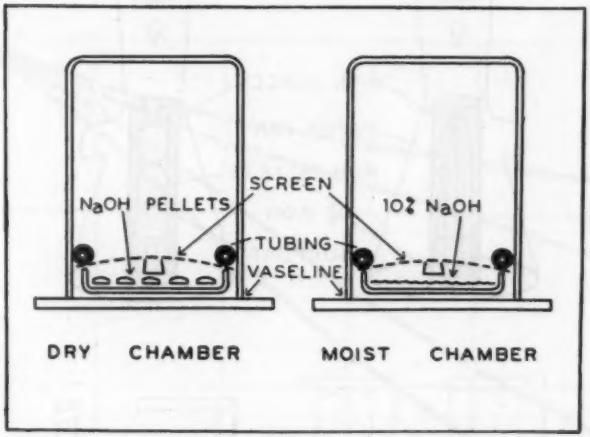

FIGURE 1. Gallon battery jars for use in following weight loss in dry and humid conditions. Flies' (upper) section separated from humidity-control section by circle of window-screen cut slightly larger than inside diameter of jar, and held between ring of pressure tubing and rim of 15 cm. petri dish. Handles attached to centers of screens.

age was much less than with a comparable solution (120 per cent). Flies were segregated from the alkali by cylindrical screens of 50 mesh brass wire. As shown in Figure 2 the paper fan and pellets were kept from touching the screen, since contact of the brass with alkali produced an additional oxygen uptake. Removal of screens when apparent oxygen uptake was maximal showed that they did not significantly impede gas diffusion. In most experiments 10 poisoned and 3 control flies were used. They were weighed individually (while narcotized) to 0.1 mg. just before transfer to the flasks. Each flask was flushed with about 1400 cc. of tank oxygen, which had no consistent effect on uptake or behavior of either control or poisoned flies. Oxygen uptake and individual behavior were recorded every 15 minutes for 6 to 12 hours, then the flies were weighed, killed in cyanide vapor, and dried. Set-up time was 10 minutes for the controls, 25 for the poisoned flies. All

gas volumes are expressed at S.T.P. Dry weights used in computing Q_{O_2}'s (mm^3/mg. dry wt./hr.) were taken as 35 per cent of initial live weights. Evidence validating various technical aspects of our respiration measurements is given in the Appendix.

Although it is usually assumed that carbon dioxide is the only gas liberated by insects, we ran six experiments (52 poisoned flies) in which half the flasks had 0.33 cc. of 5.5 per cent H_2SO_4 in the sidearms, in addition to alkali in the inset. The results indicated that no significant amounts of ammonia were produced by either control or poisoned flies in 5 hours. Likewise, in a number of experiments we interchanged flies between the "acid" and "non-acid" vessels without materially altering the apparent oxygen uptakes (Table IIB).

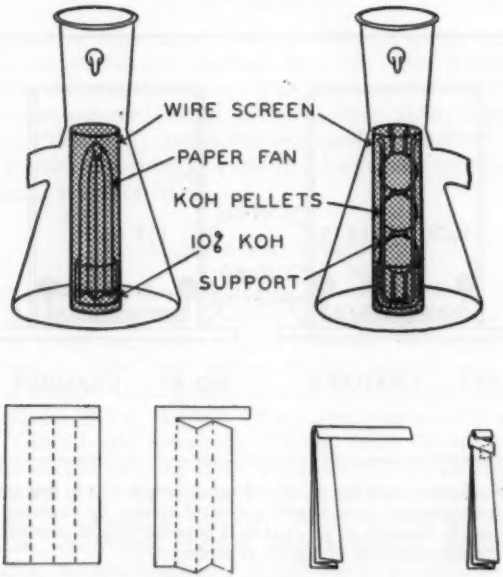

FIGURE 2. Warburg flasks modified for measuring oxygen uptake of flies under dry and humid conditions. The wires of the screening were actually horizontal and vertical rather than diagonal pellet supports made from glass rod. Other explanation in text.

Chitin was estimated by soaking weighed, hemisected flies in 8 per cent KOH for two to three weeks at room temperature, washing thoroughly, filtering on weighed paper, drying and weighing. This does not measure total skeletal material, since a large proportion of many insect cuticles is protein.

The individual variability encountered throughout the work, examples of which are seen in Tables II and III and in the standard errors indicated in Figures 4 and 9, made statistical validation of all conclusions desirable. Probability (P) values for the significance of differences between means were computed by "Student's" t test. The variability was presumably due to variations in age, feeding

and dosage, though it was not materially reduced by applying the DDT in measured individual doses in kerosene solution. Coefficients of variation in weight within groups of 13 flies from a given batch of pupae were very reasonable (6–12 per cent), and an analysis of variance showed that, with flies of comparable age, variation between experiments was no greater than within. Tests of the influence of age and of narcosis time are described later.

III. Results

A. Weight changes in live flies

Weight loss was roughly linear in all four groups during the first 10 hours (Fig. 3) although there was a three-fold difference between the maximum and

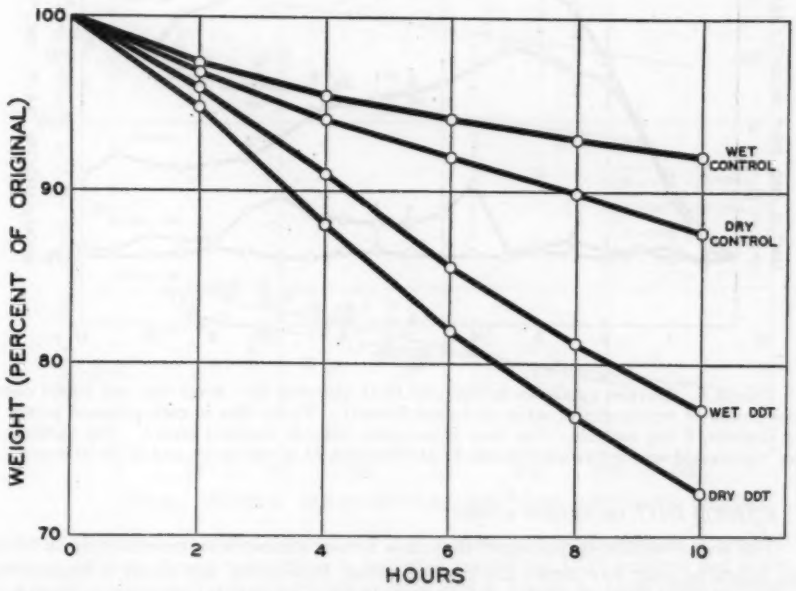

Figure 3. Live weight losses of normal and DDT-poisoned flies under dry and humid conditions. Each point represents the mean weight of 140 flies (6 experiments).

minimum group rates. Both groups in low humidity lost more weight than corresponding groups in high. The fact that the poisoned flies lost more than corresponding controls shows that DDT enhances weight loss. Overall weight loss after 10 hours becomes increasingly uncertain in poisoned groups, since some flies die, but it may considerably exceed the 30 per cent maximum found in 10 hours. In certain control groups, losses of 43 per cent occurred before death. Components of weight loss will be considered with the data on respiration and dry weight.

B. Rate of oxygen uptake of control flies

The oxygen consumption of individuals at rest was about 5 mm^3/mg. dry weight/hour (Fig. 4), a rate comparable to that in several other insects. However, occasional controls showed periods of uptake as great as those of poisoned flies (i.e. up to at least 10 times the usual control rate) coincident with periods of walking or running. Perturbations due to a single such individual out of twelve are seen in the Dry Control rate in Figure 4 (see also Fig. 7), which is otherwise not significantly different from that of the Wet Controls.

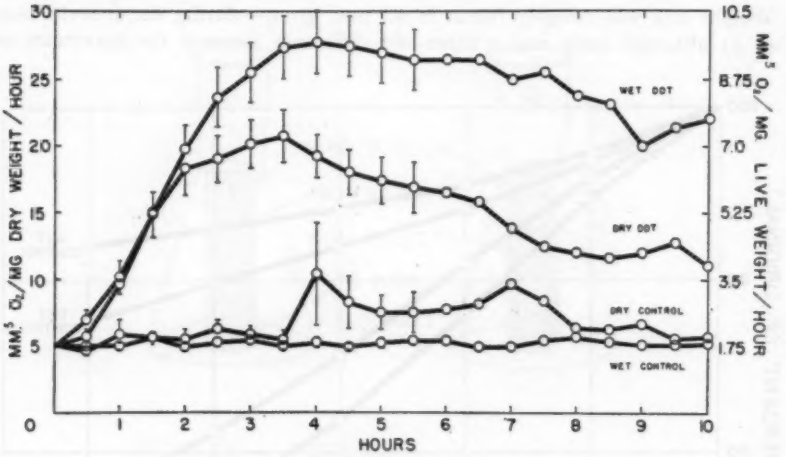

FIGURE 4. Oxygen uptake in normal and DDT-poisoned flies under dry and humid conditions (mean of representative series of 6 experiments). Thirty flies in each poisoned group, 12 dry controls, 6 wet controls. Cap lines from points indicate standard errors. The numbers of flies represented are 78 through 8 hours, 65 at 8½ hours, 51 at 9½ hours, and 35 at 10 hours.

C. Effect of DDT on oxygen uptake

The mean uptake of poisoned flies in a humid atmosphere rose steeply in about 3½ hours to over five times the control value, leveled off for about 3 hours, then declined gradually (Fig. 4). Some individuals showed brief early peaks 15–20 times the control level. The "dry" poisoned flies followed the same course for about 2 hours, but thereafter had very significantly lower uptakes ($P < 0.01$) whether calculated from initial weights or estimated weights at the times of measurement. That this is a true metabolic difference is indicated by the dry weight data given below (Fig. 5 and Table I) and by evidence (see Appendix) that oxygen uptake was measured with equal accuracy under wet and dry conditions. Accordingly, it can be concluded that a dry atmosphere partly inhibits oxygen uptake in poisoned flies. A similar inhibition has been reported in certain normal insects, but is there usually attributable to behavior differences. The unlikelihood that the effect is due to body temperature differences, such as have been observed in normal

insects at different humidities, is indicated by the lack of effect in controls. Probably the explanation lies in the rapid depletion of body water in the "dry" poisoned flies.

D. Relation between oxygen uptake and weight loss

Figure 5 shows the relations between final live and dry weights (as percentages of original live weight) and total oxygen consumptions of individual normal and poisoned flies under dry and humid conditions. The individual variability, reflected in the remarkable spread in total uptakes, is not explained by the differing durations of the six experiments in this series (8½, 8½, 10, 10½, 11 and 12 hours).

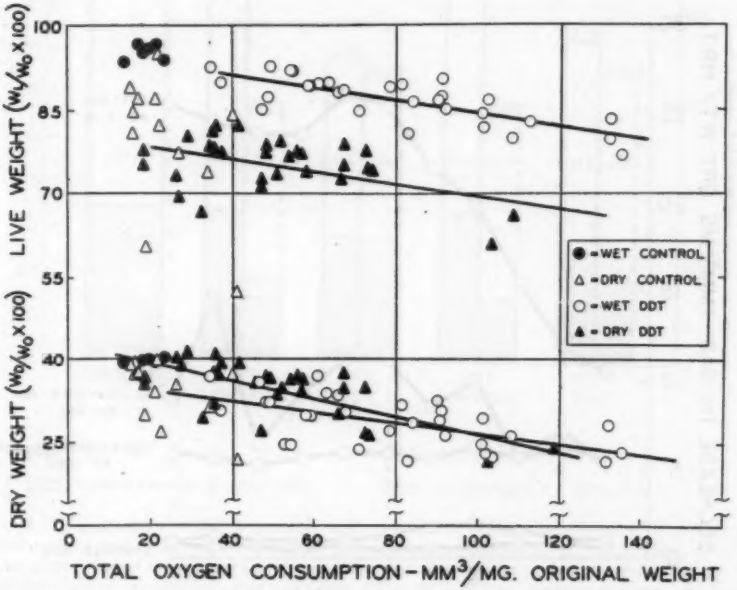

FIGURE 5. Relations between final live and dry weights (as percentages of original live weight) and total oxygen consumption in individual normal and DDT-poisoned flies under dry and humid conditions. Same experiments as those used in Figure 4. Lines fitted by method of least squares.

From Figure 5 we make the following deductions: (1) Loss of both live weight and dry weight of poisoned flies is roughly proportional to oxygen consumption. (2) The proportion of water to total solids changes little in the Wet DDT group. This means, however, that the respirable fraction of the total solid content decreases at a much higher rate than does water. (3) Total oxygen consumption is reduced by dry conditions. (4) Low humidity increases live weight loss. (5) Dry Controls seem to show a greater weight (water) loss in proportion to oxygen consumption than any other group.

E. Respiratory quotient

In experiments in which the insets of half the flasks contained 5.5 per cent H_2SO_4 instead of alkali, the net decrease in gas volume in the flasks with no absorption of carbon dioxide was almost negligible with both control and poisoned flies (Fig. 6 and Table IIA). The R.Q.'s calculated for the plateau period between 3 and 5½ hours were 0.90 for the control flies at rest, 0.93 for all controls, and 0.96 for poisoned flies. The figures indicate oxidation of a largely carbohydrate fuel. The situation is apparently different in poisoned Japanese beetle larvae, where Ludwig, by an undescribed method, obtain R.Q.'s of 0.6 to 0.8 and showed by analysis that much fat was metabolized.

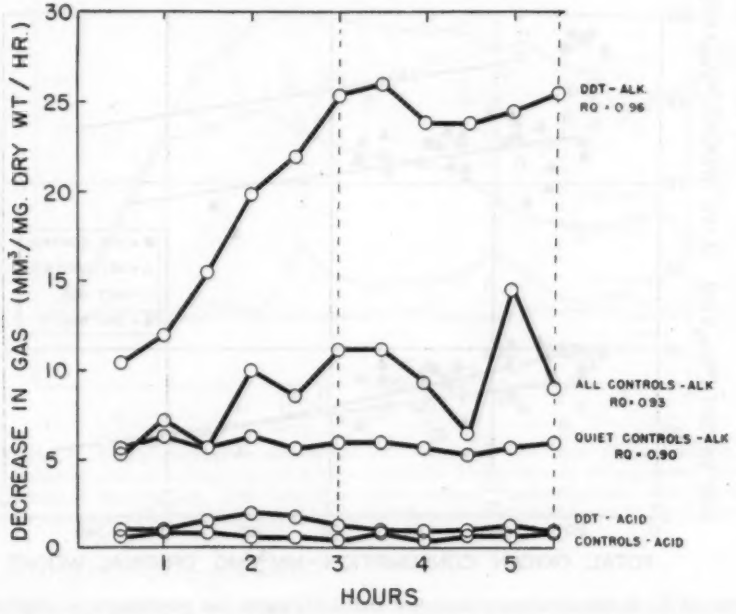

FIGURE 6. Net gas uptakes (decrease in gas volume) by normal and DDT-poisoned flies in respirometer flasks with and without absorption of carbon dioxide. Dotted lines indicate period used for calculation of respiratory quotient. Three experiments. Each DDT line based on 8 flies; alkali controls at rest, 3; all alkali controls, 6; acid controls, 9 (6 of which were at rest). Figures on 10 additional poisoned flies are given in Table IIA. The disturbing effect of activity on uptake is illustrated in the graph for all the alkaline controls.

F. Components and mechanism of weight loss

Since weight loss is nearly linear over the first 10 hours and is proportional to total oxygen consumption during the same period, rate of loss would be expected to be proportional to rate of oxygen uptake. However, uptake is far from linear in the poisoned flies, indicating that a breakdown of overall weight loss is desirable.

In Figure 7, the dotted line running through all the columns indicates the mean initial total solids (35 per cent).[1] The supposedly "irrespirable" fraction (11.9 per cent) is defined as the dry weight of Wet Control flies at death from starvation (minus the 2.5 per cent chitin fraction). The value for the "respirable solids", or material which can be used up in metabolism (20.6 per cent), was obtained by subtracting the weights of chitin and irrespirable solids from the total dry weight.

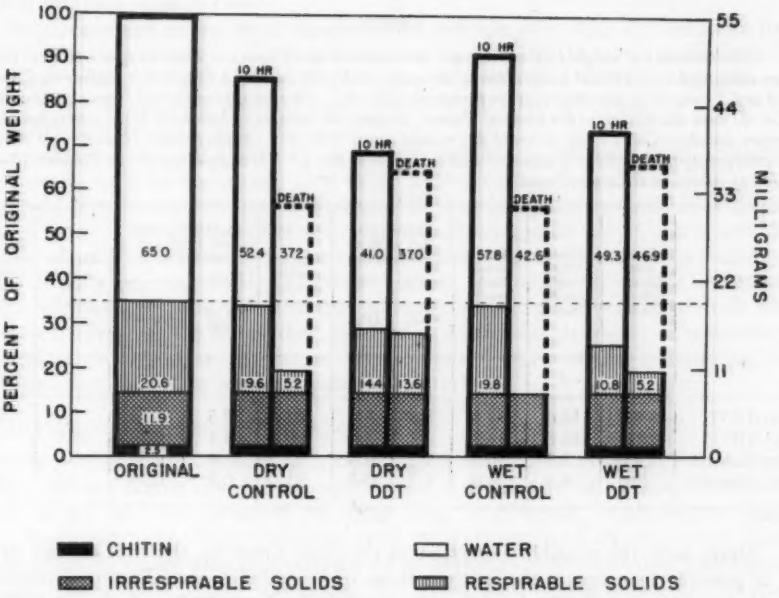

FIGURE 7. Content of water, respirable and irrespirable solids and chitin at 10 hours and at death of normal and DDT-poisoned flies in dry and humid conditions as percentages of original live weights. Based on measurements on about 860 flies in 9 experiments, including the 560 flies used for rate of weight loss measurements (Fig. 3). Water content at death subject to some uncertainty because flies did not all die simultaneously, and because the time of actual death was uncertain.

Figure 7 shows that live weights at 10 hours were in the order: Wet Control > Dry Control > Wet DDT > Dry DDT (in per cent of original live weights, 92.0 ± 1.30; 86.4 ± 1.42; 74.5 ± 1.39; 69.8 ± 1.92). All groups differ significantly ($P < 0.01$) except the Wet DDT and Dry DDT ($P = 0.08$). This indicates that both a dry environment and DDT enhance weight loss. Dry weights at 10 hours were in the order: Wet Control and Dry Control > Dry DDT > Wet

[1] This figure increases slightly with age. This explains the fact that at presumptive zero oxygen uptake, the dry weights of the flies used in the wet-dry respiration experiments (Fig. 5) average nearer 40 per cent than 35, since all the flies in this eight-day series of experiments came from the same batch of pupae.

DDT (34.2 ± 1.47; 34.0 ± 1.30; 28.8 ± 0.64; 25.2 ± 0.33), the poisoned flies having reversed their relative positions. All groups differ significantly except the controls. The data agree with the respiration experiments in indicating that DDT enhances respiration (substrate loss), and that a dry atmosphere reduces this stimulation. Furthermore, they show that DDT promotes both water loss and substrate loss though not proportionately, and that water loss is greatest in the Dry DDT flies.

TABLE I

Interrelations of weight loss and oxygen consumption at 10 hours. Total oxygen uptakes (Col. 1) were estimated by graphical integration of the areas under the curves in Figure 4. Values in Columns 2, 3 and 5 come from the weight loss experiments (Fig. 6). Dry weights estimated from oxygen uptakes (Col. 4) were calculated on the basis of glucose, as were the weights of metabolic water corresponding to oxygen uptakes (Col. 7) and observed dry weight losses (Col. 8). Reserve water (Col. 9) was obtained by subtracting the means of Columns 7 and 8 from Column 3. "Metabolic water" in Column 10 is the mean of values in Columns 7 and 8.

	Mean total O_2 uptake/fly (mm^3)	Mean total weight loss/fly (mg.)	Mean total water loss/fly (mg.)	Mean total dry weight loss/fly (mg.)		Per cent water in total loss	Metabolic water loss (mg.) calculated from		Reserve water loss (mg.)	Per cent metabolic water in total water loss
				Calc.	Obs.		O_2 uptake	Obs. dry wt. loss		
	1	2	3	4	5	6	7	8	9	10
Wet DDT	4300	14.0	8.6	5.8	5.4	61	3.5	3.2	5.2	39
Dry DDT	2820	16.6	13.2	3.8	3.4	80	2.3	2.1	11.0	17
Dry Control	1300	7.5	6.9	1.7	0.6	92	1.0	0.36	6.2	10
Wet Control	960	4.4	4.0	1.3	0.4	91	0.8	0.24	3.5	13

Since, with the possible exception of the Wet Controls, the flies do not drink, it is possible to estimate the contributions of "metabolic" and "reserve" water to total water loss, on the basis of the observed R.Q. of nearly 1. As Table I shows, metabolic water estimated from oxygen uptake and that estimated from dry weight loss agree surprisingly well for the poisoned flies, considering the crudeness of the data and the fact that they were based on quite different sorts of experiments. The estimates for the controls are less reliable because of fewer flies in the respiration experiments and proportionately larger arithmetic errors in computing dry weight losses. However, the higher observed dry weight loss in the Dry Controls is consistent with the observation that this group was more active than the Wet Controls. Table I shows also that DDT increases both reserve and metabolic water losses, and that the former was several times as great as the latter. This fact plus the fact that water accounts for most of the weight lost (Col. 6) explain why the weight loss rates of the poisoned flies (Fig. 3) showed only a slight acceleration between 2 and 6 hours, when respiration increased several-fold.

Since poisoned flies did not defecate (see section on behavior), evaporation was the only significant avenue of water loss. The increased rate of loss might be associated with an increase in the normally low cuticular permeability but seems more likely to be due simply to increased transpiration, such as would occur if DDT

caused the spiracles to remain open abnormally long. This would be consistent with the neuromuscular action of DDT, and also with the augmented metabolic rate. Unfortunately the abdominal spiracles of Phormia are too minute, and the great thoracic spiracles too inaccessible, for study without narcosis or artificial restraint. However, in the firefly *Photinus pyralis*, where the abdominal spiracles can be observed under relatively normal conditions, they open permanently in the stage of poisoning which corresponds symptomatically with the period of increasing respiration in Phormia (Buck, 1948).

Death occurred in less than one day in the average Dry DDT fly and more than eight in the Wet Controls. The water content of both Dry Controls and Dry DDT flies was around 36 per cent at death. However, the Wet DDT group still contained about 47 per cent water at death, hence death from DDT cannot be ascribed primarily to water loss.

As shown in Figure 7, the dry weights of flies which remained in the chambers until death were in the order: Dry DDT > Dry Control and Wet DDT > Wet Control (in per cent of original live weights, 28.0 ± 1.00; 19.6 ± 0.69; 19.6 ± 0.69; 14.4 ± 0.42). All groups differ very significantly except the Dry Control and Wet DDT. The Wet Control thus showed the lowest weight, instead of the highest as it did at 10 hours, and the Dry DDT the highest, instead of the next to lowest as it did at 10 hours. Hence, poisoned flies, although respiring faster than controls early in the experiment, died before they had lost as much solid material. Consequently, general substrate exhaustion cannot be the primary cause of death, assuming that poisoned flies utilize the same materials as controls.

G. Influence of hypoxia on DDT poisoning

In poisoned *Musca domestica* in air, Laug observed that oxygen uptake attained a high rate in 2 hours, then fell off rapidly to zero, coincident with cessation of movement in the fly. We observed the same thing in Phormia. Calculation showed that the cessation of uptake coincided with the exhaustion of oxygen in the flasks. Thus when the flasks were flushed with air or oxygen, the flies resumed kicking, and oxygen consumption again rose (Fig. 8). Therefore hypoxia, like cyclopropane anesthesia (Merril, Savit and Tobias, 1946), delays but does not permanently inhibit the symptoms. The symmetry of the initial peak (Fig. 8) indicates that pO_2 becomes limiting at about 90 mm. (10 per cent). This concentration was found limiting to oxygen uptake in flying *Lucilia sericata* by Davis and Fraenkel (1940), and to wing-beat frequency of flying Drosophila by Chadwick and Williams (unpublished).

In flies reviving from hypoxia, oxygen uptake often exceeded the original peak (Fig. 8), but the total uptake in the second burst of respiration showed little if any increase over the first. Since renewal of alkali (and air) after the second burst did not raise oxygen uptake above the control level, the total of the two bursts (ca. 6000 mm^3) either represented the maximum uptake of which those individuals were capable or indicated that no marked oxygen debt had been incurred.

H. Dosage effect

Laug found that house flies given 2.5 μg DDT/fly showed an earlier and smaller increase in oxygen uptake than flies given 1 μg/fly. Similarly, Ludwig's graphs indicate that peak uptake following 5 per cent DDT was higher than with either 10

per cent or 1 per cent, and that total uptakes were in the order 1 per cent > 5 per cent > 10 per cent.

To evaluate dosage effects in Phormia, oxygen uptakes were measured in culture-mates given 10 minute and 30 minute exposures to a DDT surface. As Figure 9 shows, oxygen consumption rose equally in the two groups to about three

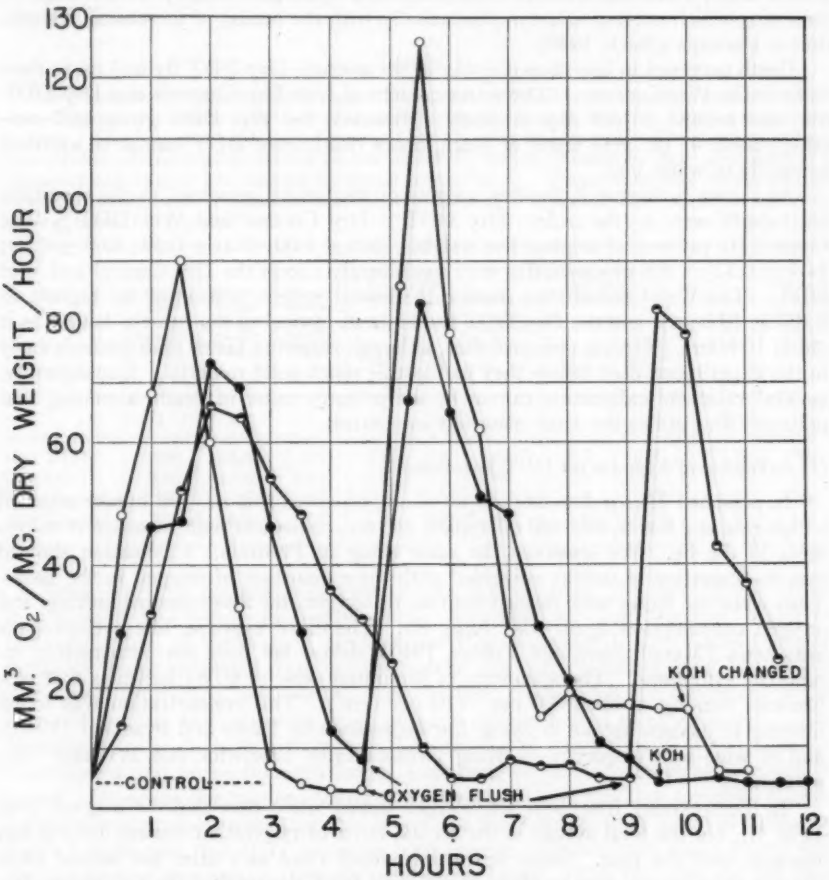

FIGURE 8. Oxygen uptakes of 3 flies respiring in air in Warburg vessels of about 15 cc. volume. Vessels flushed with oxygen at indicated points, and alkali renewed in 2 at indicated points.

times the control rate. Thereafter the more heavily dosed flies showed the lower uptake, the difference being highly significant ($P < 0.01$) after 3 hours. Therefore, since heavier doses ordinarily give a quicker and larger kill than lighter, excessive respiration is not the direct cause of death from DDT.

1. Behavior of poisoned flies

The visible symptoms of DDT poisoning, though non-specific and individually variable, are of interest in connection with the origin of the enhanced oxygen uptake. Three indistinctly separated stages of poisoning could usually be recognized. The first, lasting 15 to 30 minutes, involves locomotor hyperactivity with progressive incoördination ending in prostration. In the second, lasting ½ to 1½ hours, the flies lie on their backs, exhibiting violent spasmodic extensions and flexions of the legs, with irregular movements of proboscis and wings. Occasionally the wing movements go over into a burst of furious buzzing which lasts a minute or more.

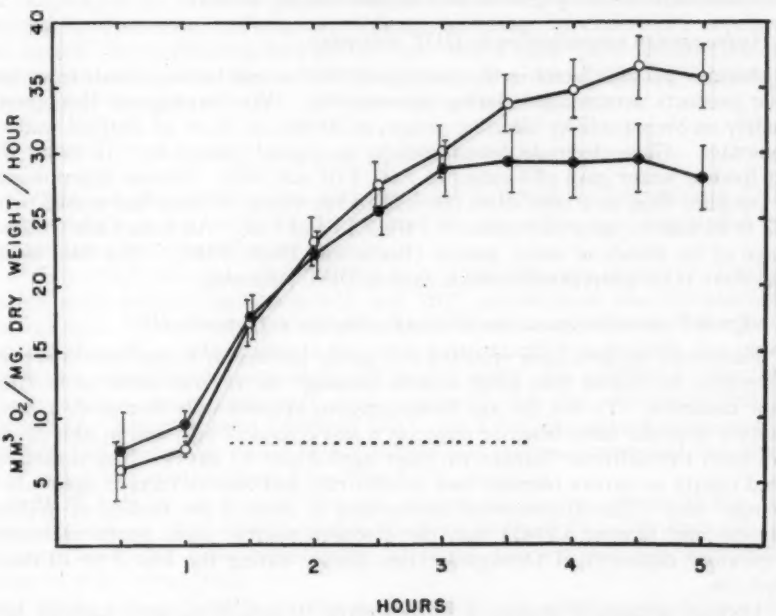

FIGURE 9. Oxygen uptake in flies given 10 minutes' exposure to DDT (open circles) and 30 minutes (closed circles). Cap lines on points indicate standard errors. Dotted line indicates control level. Upper curve 52 flies, lower curve 47 (11 experiments).

The third stage involves marked high-frequency tremors. The legs become more or less constantly flexed at the femoro-tibial joints, so that all six tarsi are brought close together. This stage continues for several hours, during which the tremors become less and less vigorous, and finally can be seen only under the microscope. At this point the fly usually voids a large drop of excrement (up to 4 mg.), the first since the start of the experiment. This adheres to the ventral surface of the abdomen and is included in the weight.

It is difficult to conclude whether or not the overt muscular activity corresponds well with the rate of oxygen uptake, although it would not necessarily do so if some

muscles were in tetany. In accord with Laug's finding, prostration occurs before peak oxygen uptake is attained. Except for the brief periods of buzzing, when very high uptakes were recorded, the first two stages are usually past before peak uptake is reached. This is somewhat surprising because the kicking of Stage 2 appears to be the most violent sustained motor activity during poisoning. However, the gradually decreasing tremors during Stage 3 correspond well with the prolonged "plateau" period of oxygen uptake (Fig. 4), and the terminal, incontinent phase, where visible activity is minimal, is always associated with a very low uptake. Correlation of activity with uptake is also indicated by the fact that the flies given a 30 minute exposure to DDT in the dosage experiments showed less violent symptoms than those with a 10 minute exposure, and appeared to die sooner.

J. Hydrogen ion concentration in DDT poisoning

Another possible factor in the toxicity of DDT would be an acidosis from metabolic products accumulated during hyperactivity. We investigated this question crudely on breis made by blending groups of 50 flies in 35 cc. of distilled water for 2 minutes. Glass-electrode determinations on control groups kept 12 hours without food or water gave pH values of 7.00, 7.03 and 7.00. Similar determinations on poisoned flies, at a time when one-half to two-thirds of them had ceased motion (12 to 16 hours), gave pH values of 7.04, 7.13 and 7.13. All values are within the range of the bloods of many insects (Boche and Buck, 1942). The data indicate that there is no generalized acidosis during DDT poisoning.

K. Age and narcosis time as variables affecting the response to DDT

Numerous workers have reported that newly hatched or young insects are more susceptible to poisons than older insects, although the reverse seems to be true in some instances. To test the age factor, oxygen uptakes were measured in five-fly samples from the same batch of pupae at 4 and 8 days of age, and in nine-fly samples from two different batches (average ages 2 and 12 days). The results indicated clearly an earlier response and greater rate and total of oxygen uptake in the younger flies. This is somewhat unexpected in view of the finding of Williams, Barness and Sawyer (1943) that the glycogen content (and hence presumptive respiratory capacity) of Drosophila rises steeply during the first 7 to 10 days of adult life.

Oxygen uptakes of groups of 15 flies given 10 and 35 minutes' narcosis before poisoning were identical over 6½ hours. This indicates that the unavoidable slight differences in narcosis time in our experiments were unimportant. However, in flies exposed for 160 minutes to carbon dioxide before poisoning, the subsequent uptake was only slightly above the usual control level, and less than one-third that of companion flies given only 5 minutes' narcosis.

IV. Discussion

From the observations that anesthetics postpone the carbohydrate depletion (Merril, Savit and Tobias), the increased oxygen uptake (Läuger et al.), and the symptoms (Bodenstein, 1946) of DDT poisoning, it has been concluded that the increased uptake is due to the increased motor activity rather than to a specific stimulation of oxidative metabolism. This view is supported by our observations

of a rough correlation between degree of activity and rate of oxygen uptake, and of high uptakes in active controls. Further evidence is afforded by the parallel between some of the physiological changes during poisoning and those during normal flight (for review see Chadwick and Gilmour, 1940; Williams, Barness and Sawyer; and Chadwick, 1947). Points of similarity are: (a) marked carbohydrate depletion, (b) R. Q. of about 1, (c) ten- to twenty-fold peak increase over resting oxygen uptake, (d) no well-defined oxygen debt, (e) oxygen becomes limiting to maximal activity at a tension of about 90 mm.

Ludwig's conclusion that carbohydrate exhaustion is the direct cause of death in poisoned Japanese beetles is opposed by the evidence of Merril, Savit and Tobias that roaches are not saved by glucose administration, nor by anesthesia sufficient to suppress symptoms and prevent carbohydrate depletion; by Chadwick's conclusion that normal Drosophila can survive for some hours after their carbohydrate has been exhausted; and by the present finding that flies dead from DDT poisoning have more "respirable substrate" left than starved controls.

V. Summary and Conclusions

Weight, oxygen uptake, and behavior were followed in normal and DDT-poisoned adults of the bluebottle fly, *Phormia regina,* under very humid and very dry conditions, with the following results:

1. Live weight loss was roughly linear over at least the first 10 hours. Poisoned flies lost more weight than unpoisoned, and "dry" groups more than corresponding "wet" groups. Water formed about 60 per cent of total weight loss in "Wet DDT" flies, and from 80 to 90 per cent in the other groups, and was probably lost almost entirely by spiracular transpiration. Metabolic water loss calculated from total oxygen uptakes was in reasonably good agreement with that calculated from dry weight loss. It is concluded that both DDT and a dry atmosphere enhance water loss; that DDT enhances loss of both metabolic and reserve water; and that loss of water is not the primary cause of death in DDT poisoning.

2. DDT induced an average five-fold increase in oxygen uptake of flies in a moist atmosphere. Individuals reached transient peaks of 15 to 20 times the control rate, associated with violent wing buzzing. The increase was significantly smaller in a dry atmosphere.

3. At the end of 10 hours, the dry weights of the four groups were in the order: Controls > Dry DDT > Wet DDT. At death the order was: Dry DDT > Dry Control and Wet DDT > Wet Control. It is therefore concluded that death is not primarily due to exhaustion of respirable substrate, assuming that the poisoned flies utilize the same materials as the controls. This conclusion is also supported by the fact that a heavier, more toxic dose of DDT produced a smaller increase in oxygen uptake than did a lighter.

4. Over a 10 hour period total oxygen uptake was proportional to both live and dry weight losses in the poisoned flies. In the Wet DDT flies, relative solid content remained approximately constant, and respirable substrate decreased at a higher rate than did water.

5. The estimated respiratory quotient was about 0.90 in controls, 0.96 in poisoned flies. Oxygen became limiting to the enhanced uptake, and to hyperactivity, at a tension of about 90 mm. Active controls reached rates of oxygen uptake com-

parable to those of poisoned flies. Overt activity showed a rough correlation with rate of oxygen uptake. Several parallels between metabolism in normal flight and physiological manifestations of DDT poisoning are pointed out. It is concluded that the increased oxygen uptake in DDT poisoning is due to the motor hyperactivity induced.

6. No ammonia production was found in either control or poisoned flies.

7. Hydrogen ion determinations on breis of normal and poisoned flies indicated that no general acidosis occurred during DDT poisoning.

8. The roles of dosage, age and narcosis in the variability of the results are discussed.

We are happy to acknowledge the advice of Drs. Edward Adolph, Leigh Chadwick, Edwin P. Laug, Kenneth Roeder, A. Glenn Richards, Julian Tobias and J. Franklin Yeager.

Appendix

Validative data on technique of respiration measurements, with tables concerning respiratory quotient, ammonia production and variability

1. Measurement of oxygen uptake at different humidities. Since oxygen uptake in Warburg flasks is almost always measured under humid conditions, it is necessary to show that no systematic physical error was involved in our measurements in a dry atmosphere. Calculation shows that the maximum possible error from the diverse ratios of water vapor to gas at the two humidities (which might produce a spurious excess in uptake in the more humid flask) is negligible. Heat of solution of the alkali pellets is likewise unimportant because no appreciable difference was seen in the apparent rates of oxygen uptake of dry and wet flies during the first two hours, when the effect should have been most pronounced, and no disturbance occurred in the Dry Control rate when moist outside air was introduced in resetting the manometers.

As an overall empirical check on our technique, we exchanged "wet" and "dry" flies at times when their rates of oxygen uptake were apparently steady (from 2¼ to 6½ hours after poisoning). In 25 flies transferred from "wet" to "dry" flasks, the mean rate of uptake in the half hour immediately following the change was 98.6 ± 4.0 per cent of that in the corresponding period previous to transfer. In 25 "dry" to "wet" transfers, the corresponding new rate was 98.3 ± 7.2 per cent of that prior to transfer. Similar results were obtained in comparing the hour previous to transfer with the hour subsequent to transfer. (The fact that uptake decreased after both exchanges merely reflects the usual gradual fall with time.) These results strongly indicate that our technique measured oxygen uptake with equal accuracy in high and low humidity, and that the difference in uptake at the two humidities is metabolic. Furthermore, it appears that the humidity factor, whatever its ultimate cause, operates only before the uptake plateau is attained.

2. Linearity and capacity of carbon dioxide absorption. In some experiments, apparent oxygen uptakes of over 2000 mm^3 per hour per 55 mg. fly were recorded for brief periods, and even the average plateau level for all the Wet DDT flies was over 500 mm^3/hr./fly (multiply the mm^3/mg. dry wt./hr. values given in the figures and tables by 19). Since these rates, and the total uptakes per fly over 8 to 10 hours, are far higher than those usually measured with the Warburg microrespirometer, it is necessary to show that absorption of carbon dioxide was not limiting in our experiments. In most of 12 instances in which two flies were put together after they had apparently reached their plateau phases of uptake (Table III) the resulting apparent oxygen uptake in the single vessel closely approximated the sum of the two previous individual uptakes (rates up to nearly 3500 mm^3/hr. were obtained). It consequently seems clear that the absorptive capacity of the alkali was adequate, as far as rate of production of carbon dioxide is concerned, and that the plateau period of uptake (Fig. 4) is not an artifact.

The calculated total carbon dioxide capacity of the alkali in the inset of the average flask was about 5300 mm^3. Although in the longest experiments (Fig. 4) the average total oxygen

uptake was only about 4300 mm³ in the most active group, several individuals approached, and (on the basis of an R. Q. of 1) a few even apparently exceeded the theoretical limit of carbon dioxide absorption. Accordingly, unless the R. Q. decreased late in the experiments (as might have happened, in view of Chadwick's finding of a great decrease in R. Q. of Drosophila after flight), it is possible that the oxygen consumptions recorded in the last hour or two of some of the experiments were actually somewhat lower than the true values. In a few flasks in which the alkali was renewed, uptake increased, but in others (for example some of those shown in Fig. 8 and Table III), it did not. Thus some individuals show a decrease in oxygen uptake like that shown in the average curve in Figure 4, even when there are no known technical limitations

TABLE II

Oxygen uptake in paired individual flies before and after exchanging experimental conditions. Data used in calculating R. Q. and evaluating inhibition by accumulating carbon dioxide (Part A) and in testing ammonia production (Part B). In part B "decrease in gas volume" is equivalent to "oxygen uptake." From 15 to 30 min. were lost in each transfer and re-equilibration.

Expt.	Fly	Original treatment	Rate of decrease in gas volume in successive ½ hr. periods before exchange (mm³/mg. dry wt./hr.)	Time of exchange (hr.)	Rate of decrease in gas volume in successive ½ hr. periods after exchange (mm³/mg. dry wt./hr.)	Rate of decrease in gas volume in successive ½ hr. periods after return to original state (mm³/mg. dry wt./hr.)
A. Exchanges between flasks with CO_2 absorption ("KOH") and those without ("acid")						
166	3	KOH	23 40 56 63	2¾	0.5 1.3 1.2 0.3	52 31 15 23
	4	Acid	0.5 0.5 0.7 1.1		9 10 12 11	0.1 0.8 0.8 0.1
	5	KOH	33 31 33 33	5	1.1 1.2 1.1	32
	6	Acid	1.0 0.7 0.9 0.7		14 18 21	0.7
	12	KOH	46 38 32 36	3¾	0.6 1.2 1.1 0.8	15 21 30
	13	Acid	1.3 1.5 1.0 1.0		19 18 16 13	0 0.4 0.8
168	3	KOH	16 49 64 70	2¾	2.4 2.1	33 15
	6	Acid	1.0 0.9 1.3 1.2		22 25	0.7 0.9 0.9
	5	KOH	29 36 38 41	3	1.0 1.1 0.7	55 62 55
	11	Acid	1.2 1.6 1.5 1.2		41 41 36	0.4 0.7 0.5
B. Exchanges between flasks with and without acid in side arms						
164	6	Acid	31 43 69	2¼	37 42	38 36 34 32
	9	Non-acid	22 44 57		87 51	20 15 18
	13	Acid	11 28 53 56	2¾	56 47 44	41 34
	14	Non-acid	16 34 36 26		32 34 30	35 34
165	9	Non-acid	27 22 31 33	4¼	28 23 12	
	14	Acid	36 36 40 42		35 33 27	

TABLE III

Oxygen uptake of paired individual flies separately and after being combined in one respirometer flask to test linearity of carbon dioxide absorption. Note that the uptakes are rates per unit weight, and thus those in the last column should be expected to approximate the means of the individual uptakes of the two combined flies, rather than their sums. Table also shows variation in "plateau" uptake levels within and between flies, variation in individual responses with time, and equivalence of carbon dioxide absorption by pellets and solution. Expts. 151 and 152, wet-dry comparisons; 162 and 164, testing for NH_3; 170–178, dosage. From 15 to 30 min. were lost in each transfer and re-equilibration.

Exp.	Fly	Original treatment	Rate of O_2 uptake in successive ½ hr. periods before combining (mm^3/mg. dry wt./hr.)				Time of combining (hr.)	Rate of O_2 uptake in successive ½ hr. periods after combining (mm^3/mg. dry wt./hr.)			
151	4	Wet	16	12	11	12	6½	13	14	15	19
	6	Wet	16	15	17	14					
	5	Dry	9	11	14	9	6½	10	7	7	5
	7	Dry	13	14	19	21					
	9	Dry	9	8	8	9	8½	30	15	16	
	11	Dry	33	35	38	39					
152	6	Wet	25	23	20	20	5½	19	18	16	
	10	Wet	18	19	18	19					
	11	Dry	20	23	16	16	5½	22	10	6	4
	13	Dry	42	35	31	28					
162	2	Non-acid	36	37	29	34	6½	30	33	34	
	10	Non-acid	26	29	33	29					
164	2	Non-acid		40	56	46	1¾	27	27	26*	
	3	Non-acid		30	47	48					
170	3	10′ DDT	40	40	42	44	5½	46	41		
	12	10′ DDT	43	39	42	39					
172	7	10′ DDT	33	42	45	53	5½	46	43†		
	12	10′ DDT	40	44	42	43					
173	11	30′ DDT	19	33	45	52	3½	50	45*		
	13	30′ DDT	59	69	55	59					
175	9	30′ DDT	49	60	74	74	4½	86	33*		
	11	30′ DDT	38	41	46	52					
178	4	10′ DDT	57	44	46	49	7	45*			
	6	10′ DDT	76	72	74	79					

* These flies were subsequently separated, and after addition of new KOH and oxygen their individual uptakes were lower than before they were combined.

† Addition of new KOH and oxygen did not change uptake.

on measurement. Adequacy of carbon dioxide absorption is also suggested by the correlation between total uptake and weights of Wet DDT flies (Fig. 5 and Table I).

3. Estimation of respiratory quotient. The method of estimating R. Q. depends on average oxygen uptake being substantially the same in the two sets of flies. This requirement would not be met if the activity of the flies in the "acid" vessels was reduced by the accumulating carbon dioxide (calculated potential maximum of 7 per cent in 3½ hours). The possibility of extensive inhibition is minimized by the facts that the net uptake in the acid vessels did not change; that no difference in behavior was observed in the two sets of flies; and that no change was produced in one of the acid vessels by flushing with oxygen. In supplementary exchange experiments (Table IIA), 4 of 5 flies originally in acid flasks did indeed have considerably lower rates of uptake than their partners originally in alkali flasks. However, the R. Q. calculated from the acid and alkali periods of the flies originally in acid flasks is 0.95, which is so close to the 0.96 derived from the data given in Figure 7 that it seems legitimate to conclude that the estimated R. Q.'s are of the correct order of magnitude.

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GROWTH OF OYSTERS, O. VIRGINICA, DURING DIFFERENT MONTHS

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Introduction

Although the American oyster, *Ostrea virginica*, is one of the most common bivalves of our Atlantic and Gulf Coasts, very little attention was given by past workers to various aspects of its growth, despite the fact that this field offers a large number of unanswered problems. For example, Moore (1898) in his voluminous article on the oyster devoted less than one page to a discussion of its growth. Churchill (1921) also confined himself to a few general statements on the growth of oysters under different environmental conditions. Yet, an understanding of the growth of oysters is of undeniable importance not only from a purely biological, but also from a practical, point of view because the oyster industry occupies one of the leading positions among the fisheries of the United States.

Nelson (1922) was perhaps the first to study more or less systematically certain phases of the growth of oysters. He measured and weighed a large group of New Jersey oysters in April and August of 1919 and again in March of 1920 noticing the increase in size and weight between the measurements. Loosanoff (1947) and his associates observed the increase in size of oysters of different ages grown during a three-year period in Milford Harbor, Connecticut. The oysters were measured once a year—in late autumn—to show the increase in size.

None of the observations made thus far was directed to study the relative increase in the size and volume of the oysters during each month of the year. This article presents the results of such studies which were carried on in Milford Harbor, Connecticut.

Winter Observations

Because it has never been satisfactorily shown whether New England oysters continue to grow during the hibernation period, experiments were carried on for three successive winters to give the needed answer.

Oysters were prepared for the experiments as follows: After the shells were cleaned of all foreign matter, the edges of the shells of each oyster were filed off to make it easier to notice new growth if any formed. The oysters were then individually numbered with small celluloid tags. Later the length, width, and depth of each oyster were measured with a vernier caliper reading to 0.1 mm. The length represented the greatest anterior-posterior dimension. The width was measured along the maximum dorso-ventral line, and the depth represented the maximum distance between the outer surfaces of the two shells.

For determining the volume of an oyster a modified Grave's (1912) method, consisting of measuring the quantity of water displaced by an oyster, was used.

The oysters were kept moist before immersion, to avoid a possible error in determining the volume, because dry shells usually absorb small quantities of water. The method was found simple but reliable, the measurements being accurate within one or two per cent.

The first experiment was begun with 80 three-year-old oysters. After the measurements were completed on December 7, 1944, the oysters were put into a large wire tray which was placed on the bottom of Milford Harbor at a depth of approximately 3 feet below the mean low water level. The water temperature on that date was 3.0° C. Soon after the tray was placed in the Harbor a layer of ice was formed and, therefore, the oysters could not be examined at frequent intervals.

The first examination was made on March 7, 1945 when the temperature of the water was still near 0.0° C. Examination of the edges of the shells showed that not in a single case was new growth formed. The final examination was made on March 20, when the water temperature was reaching 5.0° C., thus indicating that the end of the hibernation period was approaching. During this examination the length, width, depth, and volume of each oyster were re-measured and the data compared with those obtained for the same individual the preceding fall. Only three oysters died during the winter. The measurements of the living 77 oysters showed that they did not change in size or volume during the winter.

The second experiment was made during the winter of 1945–1946. A group of 120 oysters was placed in Milford Harbor at the beginning of the hibernation period and re-examined in March. All the oysters survived the winter but their shells did not increase in length, width, or depth.

The third and final experiment was conducted with 58 oysters between December 9, 1946 and March 14, 1947. In addition to the observations made during the two previous winters, the weight of each oyster was ascertained at the beginning and at the end of hibernation. The results of the March measurements showed that with the exception of one oyster which had part of its shell broken off, there was no change in length, width, depth, volume, or weight during the winter.

As a result of the observations made during three winters, we may conclude that in northern waters the shells of the oysters do not increase in size, volume, or weight during the hibernation period. However, our laboratory observations, which will be discussed in a later part of this article, showed that if the temperature of the water is kept well above the hibernation point, the oysters will continue to grow even in the middle of winter.

Observations During the Growing Period

The first experiments to determine the increase in the size and volume of the oysters during each month of the growing period were begun in the spring of 1944, but had to be discontinued in the middle of the summer because the new growth, which is almost as thin as cigarette paper and extremely brittle, broke off at the slightest touch. The experiments started in 1945 were also discontinued several months later for the same reason.

Finally, in 1946 the observations were successfully completed. On March 29, 1946, a group of 120 adult oysters was placed in a large wire tray attached to a float anchored in Milford Harbor. The float rose and fell with the tide but the position of the tray always remained approximately 3 feet below the surface of

the water. Before placing the oysters in the tray they were individually numbered, measured, and their volumes determined.

The oysters were re-examined and re-measured at the end of each month; the last measurements were made during the last days of November when the water temperature was becoming low enough to induce hibernation. To keep the oysters out of the water as little as possible during the examinations they were handled in groups of ten, because such small groups could be measured and returned to the water within a few minutes. Also, by working with small groups it was easier to avoid breaking the shells.

Of the original group of 120 oysters, 109 were alive at the end of the experiment. The conclusions offered in this article are based upon the data obtained from these survivals. The ranges in length, width, depth, and volume of the oysters at the beginning of the observations were 68.2–107.5; 50.3–85.8; 22.5–40.0 mm.; and 40–104 cc. respectively. At the end of the experiments the ranges were 85.3–135.0; 66.5–107.6; 26.4–44.3 mm.; and 65–176 cc. The mean length, width, depth, and volume of the oysters for each month are given in Table I.

TABLE I

Mean with standard error of length, width, depth and volume of oysters at the end of each month during growing period of 1946, Milford Harbor

Month	Mean			
	Length in mm.	Width in mm.	Depth in mm.	Volume in cc.
March	88.2±0.766	65.4±0.519	30.0±0.314	63.7±1.438
April	88.7±0.799	65.6±0.554	30.0±0.319	64.8±1.519
May	93.8±0.823	71.0±0.604	30.0±0.319	65.7±1.518
June	98.1±0.886	79.4±0.766	30.0±0.319	69.0±1.531
July	103.1±1.001	82.5±0.763	31.0±0.309	73.6±1.675
August	106.0±0.95	83.8±0.788	32.1±0.329	84.1±1.893
September	109.6±1.034	86.0±0.786	33.2±0.352	93.0±2.243
October	110.4±1.06	86.3±0.8	34.1±0.37	97.2±2.464
November	110.8±1.084	86.3±0.8	34.6±0.365	99.3±2.596

In estimating the monthly increases of each variate, which represented the difference between the means of each two consecutive months, the total increase for the entire growing season was taken as 100 per cent, and the monthly gains were calculated in relation to it. The results are presented in Figures 1 and 2.

As already mentioned, the oysters of our area do not increase in size or volume during the hibernation period, which extends roughly from the beginning of December until the end of March. Monthly observations showed, however, that in April the shells of the oysters begin to grow, the mean increase in length for that month constituting 2.21 per cent of the total annual increment (Fig. 1). During May, June, and July the increase in length is most rapid, being 22.57, 19.03 and 22.12 per cent respectively. Thus, during these three months the oysters achieved approximately 63.7 per cent of their annual increase in length. The percentages for August, September, October and November were 12.83, 15.93, 3.54 and 1.77

respectively. As can be seen, the months of October and November contribute but little to the total annual increase in length.

An increase in the width of the oyster shells began in April, simultaneously with an increase in length, but terminated in October, a month earlier than the latter (Fig. 1 and Table I). It was extremely rapid in May and especially in June,

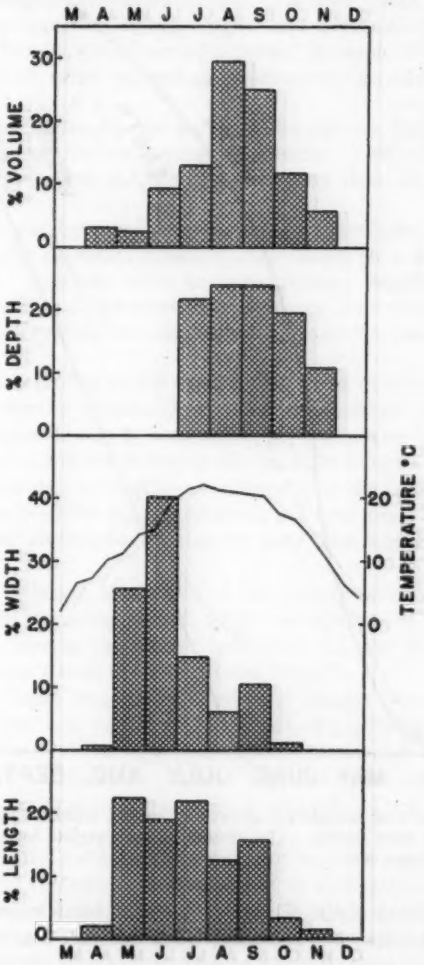

FIGURE 1. Per cent of increase in length, width, depth and volume of oysters during each month of the growing period. The total increase of each variate for the entire growing period, 1946 is taken as 100 per cent. Temperature curve is based upon semi-weekly records made at high water stages.

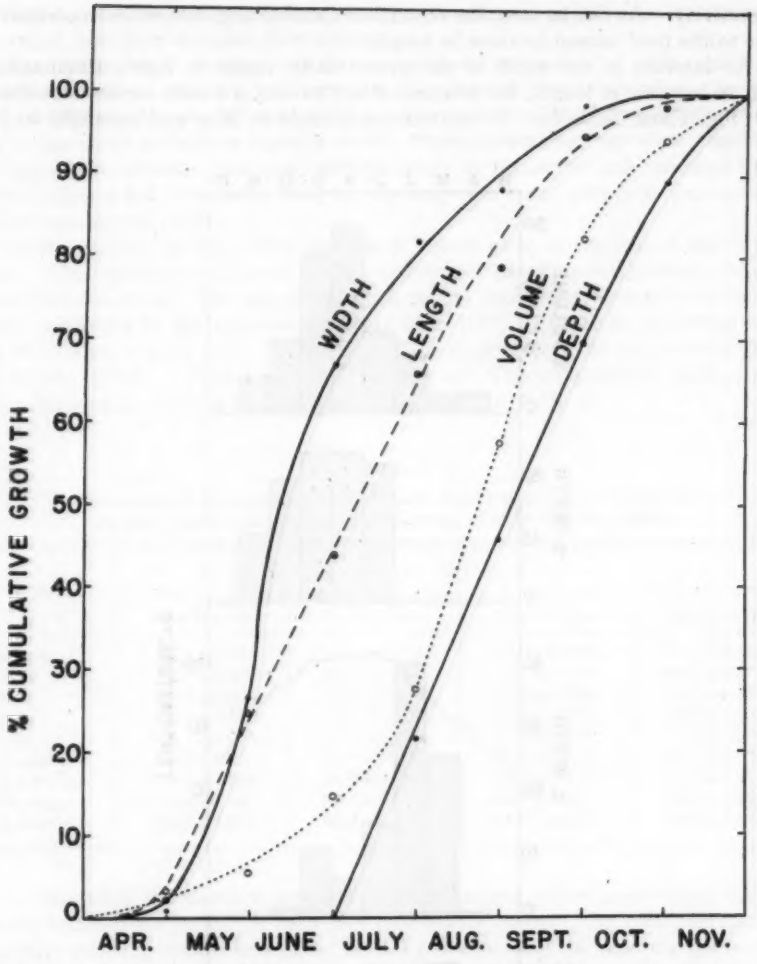

FIGURE 2. Per cent of cumulative growth in length, width, depth and volume of oysters recorded at the end of each month. The growth of each variate for the entire growing period of 1946 is taken as 100 per cent.

the latter month giving about 40 per cent of the total annual increase. In July, however, a sharp decrease was recorded. The decrease was even more pronounced during August.

Although the oysters increased in length, width, and volume during April, May, and June, the increase in the greatest depth was not appreciable until July (Figs. 1 and 2). During the first three months of the growing period the thinner parts of

the shells became thicker, but this change was not reflected in the greatest depth, or thickness, of the oysters. Nevertheless, the observations on the increase in the greatest depth are of interest because they indicate, as do the studies of the increase in volume, that an increase in depth is largely achieved during the second part of the growing period.

The increase in volume of the oysters, the same as the increase in length, continued from April through November (Figs. 1, 2 and Table I). The greatest monthly increases were recorded during August and September, these two months giving approximately 55 per cent of the total annual increase in volume. As could be expected, the period of rapid increase in volume corresponded to that of greatest depth.

The marked increase in length and width of the oysters during May and June did not materially contribute to the increase in volume. This, of course, should be expected because newly formed shell-margins are very thin, displacing only small quantities of water.

Because the experimental oysters were individually numbered, it was possible to follow the increase in size of each individual from month to month throughout the entire growing period. This was done for two variates—length and volume. As usual, when working with a large number of animals, considerable individual differences were found. Nevertheless, the individual records showed the following interesting facts:

The maximum period during which oysters may grow in Milford Harbor is approximately of eight months' duration, extending from April to November, both months inclusive. However, only a small minority, comprising approximately 3 to 4 per cent of the entire group, grew during all the eight months, while for the majority of oysters the increase in length and volume was recorded only for five, six or seven months of the possible eight. About 3 per cent grew only three months and 10 per cent showed an increase in size for only four months, which were not always consecutive.

The chief increase in length and width of the oysters occurred during the first half of the eight-months' growing period, while the increase in the greatest depth and volume took place during the second half (Fig. 1). In this respect our observations are in agreement with those of Nelson (1922).

Not all the oysters began to grow in length and volume during the first month after the end of the hibernation period. Only about 48 per cent of the entire group increased in length in April, 49 per cent in May, while 3 per cent did not start growing until June. In volume, about 29 per cent began to show an increase in April, 40 per cent in May, 27 per cent in June, while 4 per cent did not show any increase until July.

Although it is true that we are usually concerned with the average animal, nevertheless, observations and records of unusually fast or slow-growing individuals are of significant biological value and interest because they may indicate that, within what may appear to be a homogeneous population, there may be distinct fast or slow-growing races. Some of the observations on individual oysters are given in the following paragraphs.

The greatest individual increment in length for the entire season was shown by oyster no. 22 which grew from 91.8 to 129.0 mm. in seven months, an increase of

37.2 mm. The smallest increase was shown by oyster no. 98 which grew from 77.6 to 85.3 mm., an increase of only 7.7 mm. This oyster grew in length only during two months out of a possible eight.

The greatest increase in volume for any individual was made by oyster no. 5, which increased from 104.0 to 176.0 cc., a total of 72 cc. in seven months. Oyster no. 17 showed an increase of only 8 cc., growing from 57.0 to 65.0 cc. in five months.

Individual records also made it possible to ascertain the maximum increase in length or volume of the fastest growing oysters for every month of the season (Table II). The largest monthly increase in length was made by oyster no. 90, which during July increased 15.2 mm., representing an increase of 17.9 per cent over the total length recorded at the end of June. During November the fastest growing oyster increased its length only by 3.4 per cent.

TABLE II

Greatest monthly increases in length or in volume shown by individual oysters. April–November, 1946. Milford Harbor

	April	May	June	July	Aug.	Sept.	Oct.	Nov.
Length								
Oyster no.	81	104	78	90	82	21	22	26
Old size in mm.	78.0	90.3	94.3	84.7	110.9	119.1	121.5	114.6
New size in mm.	82.5	100.8	105.6	99.9	117.9	129.1	129.0	118.5
Increase in mm.	4.5	10.5	11.3	15.2	7.0	10.0	7.5	3.9
% Increase	5.8	11.6	12.0	17.9	6.3	8.4	6.2	3.4
Volume								
Oyster no.	7	1	59	78	34	22	49	9
Old volume in cc.	73	78	73	72	79	98	106	128
New volume in cc.	78	82	84	87	99	127	122	136
Increase in cc.	5	4	11	15	20	29	16	8
% Increase	6.8	5.1	15.1	20.8	25.3	29.6	15.1	6.3

The greatest monthly increment in volume was made in September by oyster no. 22, which increased 29 cc. or 29.6 per cent over the volume recorded at the end of the preceding month.

It is significant that the greatest individual increases in length occurred during May, June, and July, that is, during the months when the group as a whole grew in length most rapidly. For the volume, both the greatest individual and group increments were noted in August and September (Fig. 1, Table II).

The records also show that the per cent of oysters increasing in length or volume varied considerably during different months. The increase was most common during July and August when almost all the oysters showed it, and the least noticeable in April and November.

Observations made on monthly growth of oysters in Milford Harbor suggest the following conclusions and deductions: Growth of the oysters, taken as a group, continued throughout the period extending from April to November, both months in-

cluded, without definite interruption during the spawning season. This observation is in agreement with the conclusions of several investigators working with other lamellibranch mollusks. For example, Belding (1912) found that the hard-shell clam, *Venus mercenaria,* grows very fast during July and August when its spawning is in progress. During these two months the clams show approximately 45 per cent of the total annual increase in the length of the shell. Belding (1931) also found that the soft clam, *Mya arenaria,* which in Massachusetts waters spawns during June, July, and August, shows during these three months approximately 55 per cent of the annual increase in the length. Coe (1945, 1947) observed that the California bay-mussel, *Mytilus edulis diegensis,* also grows during the spawning period.

In other lamellibranchs, however, the rate of growth may be appreciably diminished during the spawning season. Belding (1910) noticed such a decrease in the bay scallop, *Pecten irradians.* Coe (1947) thinks that the decrease in monthly increments in length of the Pismo clam, *Tivela stultorum,* in August is due "to the requirements of the reproductive system and the successive acts of spawning."

In the case of oysters, Nelson (1922) found that *O. virginica* of the New Jersey coast grows rapidly until the spawning period but more slowly thereafter, while Orton (1935) observed two main periods of shell growth of *Ostrea edulis,* one in spring and one in autumn.

A very rapid increase in the mean length and width of the oysters of Milford Harbor occurred during May and June, i.e., during the period of most active gametogenesis for the oysters of this region (Loosanoff, 1942). Apparently, the process of development and accumulation of gametes did not interfere with the growth of the shell, at least as far as the increase in length and width was concerned. This conclusion is well supported by observations on oysters which are conditioned every winter in our laboratory to develop ripe eggs and spermatozoa (Loosanoff, 1945). The oysters are brought from the beds in the hibernating state and after being kept at room temperature for several hours are placed in trays with running warm water. In a month or less, depending upon the temperature of the conditioning trays, the oysters are ripe. Yet, during this period of extremely active gametogenesis the majority of the oysters grow rapidly in length and width, forming new shell-margins which quite often are over 1.0 cm. This proves, of course, that gonad development and rapid growth of shell may proceed simultaneously.

Mass spawning of the experimental oysters was observed during the last few days of June. There is no doubt that these oysters continued spawning during July and that many of them completed spawning during that month. The latter point was ascertained by opening Milford Harbor oysters not used in the experiment. Therefore, we concluded that since July was the month of most active spawning and since the increase in length during that month was very rapid, it is apparent that the spawning activities did not sharply affect the rate of increase in the shell length. In this respect our conclusions differ from those of Orton (1928) who reported that the rate of growth of the European oyster, *O. edulis,* is considerably slowed down during the breeding period.

It may be tempting to explain the slowing of the growth in width of our oysters during July by ascribing it to the spawning activities. Such an explanation, however, does not appear to be very conclusive because three other variates showed an increase during that month (Fig. 1). Even if the rate of increase in width during

July was considerably slower than that observed in June, it still was comparatively rapid, occupying the third position among the eight months of the growing season. Moreover, our laboratory experiments on the conditioning of oysters for spawning in the winter gave us additional proof that spawning does not stop, or seriously decrease, the rate of shell growth. For example, in February, 1949, a group of 105 oysters was brought from Long Island Sound, where the water temperature was below 5.0° C., and after being measured was placed in warm running sea water at 25.0° C. At the end of the ninth day at this temperature the oysters spawned. Some of the spawning oysters had already at that time a new shell growth which measured over 1.0 cm. After spawning, the oysters continued to form a new shell for some time.

The slowing down of the rate of growth in length and width during August also should not be attributed to the spawning activities because of the considerations presented above. Furthermore, spawning was almost completed during July. Perhaps the slow growth could be more logically associated with the post-spawning stage, during which emaciated oysters are, presumably, not in condition to divert much of their energy into building new shell substance. This assumption is again easily invalidated because of the pronounced acceleration in the increase in volume noticed in August and in early September (Fig. 1).

There are some indications of possible physiological antagonism between the growth of oysters and the process of accumulation of glycogen in their tissues, a phenomenon commonly known as "fattening of oysters." In our waters, chief accumulation of glycogen in the meats of oysters occurs between the completion of spawning and until hibernation, thus covering a period of approximately three months, namely, September, October, and November. During this period the rate of increase in size and volume of oysters progressively diminishes (Figs. 1 and 2). Whether this decrease is due to the true antagonism of the different physiological functions, or merely reflects the changes occurring in the surrounding water, remains at present undetermined.

The changes of the water temperature and the monthly rates of growth of oysters showed only a partial relationship. It is true that the increase in length and width of the shells recorded in April, May and June was accompanied by a steady rise in temperature (Fig. 1). In July, however, the rate of increase in the width markedly decreased, although the temperature remained above 20.0° C., but such a presumably favorable condition was not reflected in the rate of increase in length and width. The comparatively slow rate of growth in length and width observed during October cannot be explained by the unfavorably low temperature, because during that month the average temperature was not lower than that recorded for May and the early part of June when the shells grew so rapidly.

A much clearer relationship was found between the monthly increments in volume and the changes in water temperature (Fig. 1). In spring and early summer the monthly increments increased simultaneously with the increase in temperature. The period of the most rapid monthly increases in volume roughly corresponded to the period of maximal seasonal temperature, while in the fall both showed a gradual decline.

In connection with these studies it was thought desirable to determine by experimental means the rate of growth of groups of oysters kept at different temperatures. This was done in the winter time because it was easier then to maintain in the lab-

oratory the desired temperatures merely by mixing definite quantities of cold and warm running sea water.

The warm sea water system, which is operated in our laboratory during the cold season, is regulated by a series of thermostats which control the temperature of the outflowing water. The temperature of our cold water is also very uniform. Therefore, in the winter time water of any temperature within the range of about 5.0° to 35.0° C. can be had by using constant level jars of cold and warm water and by regulating by stopcocks the flow from these jars into a mixing chamber until the desired temperature is obtained. From the mixing chamber the water is flowed into the trays or aquaria containing the oysters.

In the middle of February a shipment of four-year-old oysters, consisting of individuals of approximately the same size, was brought from the beds of Long Island Sound and placed for several hours in sea water of about 8.0° to 9.0° C. to let the oysters come out of hibernation. Then they were divided at random into four groups each containing 105 animals. After determining the average length and width of each group (Table III), the oysters were placed in trays with running water the temperature of which was brought up and then steadily maintained at approximately 10.0°, 15.0°, 20.0° or 25.0° C. All the trays were receiving the same quantity of water.

TABLE III

Average increase in length and width of oysters kept at temperatures of 10.0, 15.0, 20.0 or 25.0° C. from February 15 to March 16, 1949

Temperatures	10.0° C.		15.0° C.		20.0° C.		25.0° C.	
	L.	W.	L.	W.	L.	W.	L.	W.
Original measurements 2/15/49	92.1	70.3	91.5	68.6	92.5	70.1	94.0	70.1
Final measurements 3/16/49	93.4	71.3	99.9	76.0	100.1	77.3	98.5	73.2
Increase in mm.	1.3	1.0	8.4	7.4	7.6	7.2	4.5	3.1
% Increase	1.4	1.4	9.2	10.8	8.2	10.3	4.8	4.4

A month later the oysters were again measured (Table III). The 15.0° C. group grew best, showing at the end of the experiment an increase of 9.2 per cent in length and 10.8 per cent in width of shell. The maximum increase in length shown by the fastest growing oyster of this group was 21.0 mm. The growth of oysters kept at 20.0° C. was almost as fast as that of the 15.0° C. group. However, the 25.0° C. group grew much more slowly than the two above mentioned, and the 10.0° C. group showed only a slight increase in size. The maximum increase in length attained by the fastest growing oysters of the 25.0° and 10.0° C. groups was 14.0 and 5.0 mm. respectively.

In the 15.0° C. group all the oysters showed new growth and in 20.0° C. only one individual did not form new shell. In the 10.0° and 25.0° C. groups, however, many oysters did not grow.

Examination of the new shell growth showed that its character was different in the different groups. In the lowest group the new shell was, at the end of the experiment, still transparent, soft and flexible. In the 15.0° C. group, however, the

new shell substance was already becoming harder and more brittle, and only the most recently formed part, confined to the edges of the shell, was still soft and flexible. This condition was even more pronounced at higher temperatures but, nevertheless, even in those groups many oysters were still forming new growth during the last days of the experiment.

Thus, under the conditions under which the experiment was run, the oysters grew most rapidly at temperatures of 15.0° and 20.0° C. Therefore, the optimum temperature range for their growth was either confined between these two temperatures or, what is more probable, extended a degree or two outside these two limits giving a range from approximately 13.0° to 22.0° C. It is interesting that the rapid increase in length and width shown in the spring and early summer by the oysters grown in Milford Harbor took place during May and June, in other words, when the water temperature was within the range given above (Fig. 1).

Our laboratory observations on the growth of oysters at different temperatures were, however, of too short a duration to find whether, if the experiment had been continued for several more months, the growth of each group would have proceeded at its original rate or would have shown some important changes. For example, it is possible that if the experiment had been prolonged, the rate of growth of the fast growing groups of 15.0° and 20.0° C. would have gradually decreased and, perhaps, eventually stopped, while the growth of the 10.0° C. group would have proceeded at the same or even at a somewhat faster rate than that shown during the first month of observation. It is planned to find the answer to this question in the near future.

With our present knowledge, it is impossible to estimate accurately the effect of food upon the growth of oysters. In a basin, such as Milford Harbor, where the tidal currents are swift and where the difference between high and low water levels may be as much as 9 feet, the quantity and quality of the material suspended in the water flowing over the oysters changes continuously. Even if it were possible to collect samples continuously, such samples would be of only limited value because many forms composing nanno and ultraplankton disintegrate almost immediately after collection. Thus, even if the quantity of material suspended in the water could be somehow determined, the quality of part of it would remain unknown.

Perhaps the greatest handicap facing the students of the role of food upon growth and other phases of the physiology of oysters is our lack of definite knowledge as to what really is the food of these mollusks. A full discussion on this subject is not the purpose of this article—those interested are referred to a summary published recently (Loosanoff and Engle, 1947). Briefly, however, while one school of investigators assumes that living plankton is the main ingredient of the oyster diet, the second school led by Coe (1945, 1947) is of the opinion that most of the nutrition of oysters, clams, mussels and other filter-feeding bivalve mollusks is derived from the intra-cellular digestion of particles of detritus originating from the disintegrated cells of marine animals and plants. Coe's conclusions appear to be well supported but, nevertheless, the issue is still debatable and not finally solved. As long as it remains in this stage, and as long as the value of different components of plankton and detritus are undetermined, it will remain impossible to formulate intelligently the relationships between the quantities or qualities of food present in the water over the oyster beds and the various aspects of the physiology of oysters or other mollusks closely related to them.

The difficulties of solving these problems are further complicated because oysters, and probably some other lamellibranchs, can feed efficiently only when the concentration of plankton (Loosanoff and Engle, 1947) or turbidity-creating substances as detritus or silt (Loosanoff and Tommers, 1948) do not exceed certain thresholds. If such thresholds are exceeded, the normal existence of mollusks becomes impossible. Thus, in addition to determining what organisms or materials constitute oyster food, it will also be necessary to determine their optimum concentrations in relation to the feeding and growth of oysters.

It should also be mentioned that a rapid increase in length and width of shell does not necessarily indicate that the oysters are growing under favorable conditions. For example, on several occasions at Milford Laboratory the oysters discarded after being used in the experiments were crowded in small aquaria through which only a trickle of water passed. Yet, within a short time some of them showed new shell growth. This growth was formed despite the fact that the oysters were not receiving enough food and that the water in which they were kept contained large quantities of waste products. Similar observations were made on oysters kept in heavy concentrations of micro-organisms, such as Chlorella and Nitzschia, which interfered with the normal feeding. The oysters eventually died (Loosanoff and Engle, 1947) but, nevertheless, even if their meats were emaciated, new shell growth was forming shortly before their death. These observations suggest that the factors involved in the growth of oysters are rather complex and at present not well understood.

The data and the conclusions on the monthly increase of oysters offered in this article are based upon only one year of observations. It is possible that during some years, when conditions are unusually favorable and the water temperature is considerably above normal during March or December, a slight increase in the size of the shells may be noted during these months. It is also possible that the monthly increases of the variates in different years would differ somewhat from those shown in our Figure 1. Nevertheless, it is believed that such variations would not basically change the trend of growth during the year.

Summary

1. The oysters grown in Milford Harbor did not increase in size, volume or weight during the hibernation period. However, if by some artificial means the temperature of the water is kept above the hibernation point, the oysters will continue to grow in the laboratory even in the middle of winter.

2. The maximum period during which oysters may grow in Milford Harbor is approximately of eight months' duration extending from April to November, both months inclusive. Only a small minority comprising approximately 3 to 4 per cent grew during all the eight months, while the majority grew only for five, six, or seven months. Some oysters did not start growing in length until June, and in volume until July.

3. The increase in length was most rapid during May, June, and July, representing 22.57, 19.03 and 22.12 per cent respectively of the total annual increment. The growth in width was especially rapid in June, giving 40 per cent of the total annual increase. The increase in the greatest depth was not appreciable until July.

4. The increase in volume continued from April through November; the greatest

monthly increases were recorded during August and September; these two months combined gave approximately 55 per cent of the annual increase in volume.

5. The increase in size was most common during July and August, when almost all the oysters showed it, and least noticeable in April and November.

6. The process of gametogenesis did not interfere with the growth of the shell, at least as far as the increase in length and width was concerned.

7. The spawning activities did not adversely affect the rate of increase in length and in volume.

8. The chief increase in length and width of the oysters occurred during the first half of the growing period, while the increase in depth and volume was most pronounced during the second half.

9. Changes in the rate of growth in length and width showed only partial relationship with changes in the water temperature. However, a rather definite relationship was found between the changes in the rate of increase in volume and changes in the water temperature.

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THE PREZONE PHENOMENON IN SPERM AGGLUTINATION

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The agglutination of the sperm of certain marine invertebrates by a substance known as fertilizin from the jelly hull of the eggs of the same species resembles serological reactions. This was first pointed out by Lillie (1919), and the concept has been expanded more recently by Tyler (cf. Tyler, 1948) on the basis of various features of the interactions of fertilizin and antifertilizin. One of these features is the occurrence of the zone phenomenon.

The zone phenomenon is typical of ordinary serological reactions (see Marrack, 1938). It is typified by the occurrence of maximum amounts of agglutination or precipitation when antigen and antibody are mixed in certain proportions. Increasing or decreasing the amount of antigen, or (in some cases) of antibody, above or below the optimum value results in decreasing amounts of agglutination or precipitation. The zones are also revealed by the occurrence of more rapid reactions (agglutination or precipitation) when the reagents are mixed in the proportions of the optimum zone than when mixed in other proportions. The mutual multivalence theory of antigen-antibody reactions proposed by Marrack (1938) and by Heidelberger (1939) offers an interpretation of the occurrence of zones. According to this theory, antigen and antibody molecules are both multivalent with respect to the mutually complementary groups by which they combine, with two or more such groups on each molecule. This would permit the formation of large aggregates of antigen and antibody. If the antigen were a cell surface, as in agglutination reactions, the process would result in agglutination. This hypothesis has received substantial support from the recent work of Pauling et al. (1944). Tyler (1940b) suggested that this line of reasoning could be applied to the agglutination of sperm by homologous fertilizin.

The occurrence of a prezone in an agglutination reaction may be interpreted in at least two ways according to the "framework" theory of antigen-antibody reaction. The prezone in agglutination reactions occurs in the region of highest agglutinin concentration; thus there would be such an excess of agglutinin molecules that the combining groups on the surface of the cell would each bind a separate agglutinin molecule, and no single agglutinin molecule would thus be likely to combine with more than one cell. This would result in little or no agglutination in very high concentrations of agglutinin and in increasing amounts of agglutination as the agglutinin concentration is lowered.

Another interpretation would be that "univalent agglutinin" molecules (agglutinin molecules with only one specific combining group each) are present along with the multivalent agglutinin molecules. Thus in regions of high agglutinin concen-

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tration, the univalent agglutinin would be able to combine with many of the specific combining groups on the agglutinogen surface and thus inhibit agglutination by the multivalent agglutinin. In any particular case, one or the other or both interpretations may apply.

Tyler (1940a) noted the occurrence of the zone phenomenon, as exhibited primarily by rates of agglutination, when sperm of the limpet Megathura were mixed in various proportions with homologous egg water. With a given amount of sperm, for example, the most rapid agglutination does not occur with the strongest egg water but with lower concentrations. Further decrease in egg water concentration, of course, gives slower and less extensive reactions until the end point of no visible reaction is reached.

It was of interest to investigate the possible occurrence of the zone phenomenon in another group of animals, the sea urchins, which differs from the molluscs in certain details of the agglutination reaction of sperm with egg water (see Tyler, 1940a, 1941). Also it seemed desirable to examine this on the basis of degree of agglutination rather than simply on the basis of rate of reaction.

Materials and Methods

Sperm and fertilizin of the sea urchin *Lytechinus pictus* were used for the experimental work. The testes were carefully removed from the animals to avoid contamination with body fluid, and the "dry" sperm was allowed to extrude. This was filtered through bolting silk and stored in small sealed flasks at 8° C. until used. For testing purposes, a 1 per cent suspension of the sperm in filtered sea water was prepared. The fertilizin solution was prepared by allowing a thick suspension of eggs to stand for several hours, centrifuging it and using the supernatant, or by extraction with acid sea water as described by Tyler (1940b). No consistent differences were noted in the action of the fertilizin prepared by these two methods. For observing the agglutinating activity of the fertilizin preparations, serial dilutions in filtered sea water with a final volume of 1.0 ml. were prepared in 10 × 75 mm. test tubes, and 0.1 ml. of 1 per cent sperm suspension added. The tubes were agitated to mix the contents, and the degree of agglutination read macroscopically at 15 minutes after adding the sperm. The degree of agglutination was expressed in the customary 0 to 4 + terminology.

Experimental

In general, when constant amounts of homologous sperm are added to serial dilutions of fertilizin, the degree of agglutination observed is proportional to the fertilizin concentration. It should be pointed out that Lytechinus sperm does not show the rapid reversal of agglutination characteristic of the *Strongylocentrotus purpuratus* sperm plus fertilizin system. In the case of Lytechinus, the agglutination may persist for several hours, although this varies with sperm from different individuals. Extremely fresh sperm often shows a very low degree of agglutination when fertilizin is added, but after aging for several hours in the refrigerator, the degree of agglutination usually increases.

With some samples of Lytechinus fertilizin, it was noted that there was little or no agglutination in the highest fertilizin concentrations, although good agglutination

occurred with lower concentrations. This resembles the prezone observed occasionally in a bacteria plus specific antiserum system, such as that described by Coca and Kelley (1921) for certain antisera against *Klebsiella capsulata*.

Wiener (1944) suggested that the prezone in the agglutination of Rh positive erythrocytes by certain anti-Rh sera was due to the presence of what he called "blocking antibodies," i.e., anti-Rh antibodies capable of specifically combining with Rh positive cells but unable to agglutinate them. The fact that the antibody had actually combined with the cells was established by showing that these cells could not be subsequently agglutinated by normal anti-Rh antisera.

It has been shown that treatment of fertilizin with heat, x-rays, and ultraviolet light (Tyler, 1941; Metz, 1942) converts it into the "univalent" form, that is, a form where it can no longer agglutinate the sperm, although it is still capable of combining specifically as may be shown by inhibition tests. This probably is due to the splitting of the fertilizin molecule into fragments containing only one effective group each.

In the first experiments conducted in the present work, the fertilizin was irradiated with ultraviolet light (source described by Spikes, 1944) to produce univalents. Later it was found that irradiating the fertilizin with visible light in the presence of a photo-sensitizing dye (eosin) according to the first method described by Tyler (1945) gave better results.

TABLE I

Degree of agglutination in the dilution of irradiated fertilizin indicated

Hours of irradiation	Titer of fertilizin									
	0	1	3	9	27	81	243	729	2187	6561
0	0	+++	+++	++++	++++	++++	+++	+	±	0
4	0	+	++	+++	+++	+++	++	+	±	0
20	0	0	+	++	+++	+++	++	±	±	0
26	0	0	0	+±	++	+++	++±	+	±	0
40	0	0	0	0	+	++±	++±	+	±	0
65	0	0	0	0	0	++±	+++	0	0	0

It was found that by using preparations containing univalent fertilizin along with the agglutinating type, the width of the prezone could be increased to a remarkable degree without appreciably affecting the beginning of the postzone. This is shown in Table I, which indicates the degree of agglutination resulting when constant amounts of sperm were added to serial dilutions of fertilizin irradiated with 750 foot candles of daylight-type fluorescent light in the presence of 0.2 per cent eosin for the number of hours indicated. If fertilizin which had been irradiated until it no longer produced any agglutination was added to normal fertilizin, a prezone was also produced.

Explanations for this induced prezone in terms of the "framework" theory of agglutination may now be suggested. If part of the molecules in a sample of fertilizin are converted into the univalent form by the irradiation, serial dilutions of this made, and constant amounts of sperm added to each dilution, there may be sufficient univalent fertilizin in the region of high fertilizin concentration to combine

with all of the combining groups of all of the sperm present, and thus prevent agglutination. In setting up the serial dilutions the fertilizin solution is successively diluted, while the amount of sperm added to each dilution remains constant. Therefore, a dilution would be reached where there would no longer be sufficient univalent fertilizin to combine with all of the sperm, thus leaving some over to be agglutinated by the normal (multivalent) fertilizin present.

An alternative suggestion would be that the irradiation breaks the fertilizin molecule into a number of fragments which are still multivalent. Then when the sperm were added there would be such great competition for the combining groups on the sperm surface that it would be difficult for one fertilizin fragment to combine with more than one sperm. This again would result in a lower degree of agglutination in the region of high fertilizin concentration. There is at least one serious objection to this latter explanation, however. If the number of multivalent fragments was increased by the irradiating process, it would be expected that the endpoint of the agglutination reaction would occur at a higher dilution of the fertilizin than before the irradiation. An examination of the data in Table I, however, shows that the endpoint was moved in, rather than outward, to a region of higher fertilizin concentration.

From either of the above interpretations it could be predicted that multivalent fertilizin should be present in the tubes in the prezone region. This was shown to be true by successive absorptions with small quantities of sperm. The first few quantities of sperm added to the prezone dilutions were not agglutinated, but a point was soon reached where added sperm was strongly agglutinated. Presumably at this point (according to the first explanation suggested above) all of the univalent fertilizin was absorbed out so that the multivalent fertilizin present was able to combine with and agglutinate the sperm. The results of a typical experiment of this type are shown in Table II.

TABLE II

Degree of sperm agglutination in various dilutions of 65 hour irradiated fertilizin after successive absorptions with sperm

Number of times absorbed with 0.2 ml. of 2% sperm	Titer of fertilizin						
	0	3	9	27	81	243	729
1	0	0	0	0	++±	+++	+
2	0	0	0	++	+++	±	0
3	0	0	±	++++	+	0	0
4	0	+	++	++	±	0	0
5	0	+++	+++	±	0	0	0
6	0	+++	++++	0	0	0	0
7	0	+++	++	0	0	0	0
8	0	++++	0	0	0	0	0
9	0	++++	0	0	0	0	0
10	0	++++	0	0	0	0	0
11	0	++++	0	0	0	0	0
12	0	++++	0	0	0	0	0
13	0	++	0	0	0	0	0
14	0	±	0	0	0	0	0
15	0	0	0	0	0	0	0

The work reported above is regarded as further evidence of the similarity of the reactions between the specifically combining substances of sperm and eggs to serological reactions.

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AN ELECTRON MICROSCOPE STUDY OF THE EGG MEMBRANES OF MELANOPLUS DIFFERENTIALIS (THOMAS) [1, 2]

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Extensive research has been carried out on the origin and the physical and chemical properties of the membranes surrounding the egg of the grasshopper *Melanoplus differentialis* (Thomas). The results of Campbell (1929), Jahn (1935a, 1935b, 1936), and Cole and Jahn (1937) seem most helpful in understanding its physical and chemical nature, while Slifer (1932, 1937, 1938a, 1938b), and Slifer and King (1934) give a clear picture of the structural relations of the egg membranes during embryonic development. It is the purpose of this paper to present results obtained from a study of the egg membranes with the aid of the RCA Electron Microscope, Model EMU-2B.

The outer membrane of the grasshopper's egg, the chorion (about 20 μ thick), is secreted by the cells of the maternal ovariole epithelium which enlarge during yolk deposition. Investigators seem to disagree regarding the formation and continuity of the vitelline membrane which lies just inside the inner surface of the chorion (Slifer, 1937). Since this membrane appears to become fragmentary as soon as embryonic development begins, it was not studied with the electron microscope.

At the time of laying, the egg, which has broken away from the ovariole epithelium, passes down the oviduct and out of the ovipositor into a pod made up of from 10 to 150 eggs. During the development of the blastoderm and its differentiation into germ band and serosa, very little change occurs in the egg membranes. The serosa cells migrate peripherally and completely surround the yolk and germ band by the fifth day (at 25° C.). They appear just inside the chorion as large, flat cells, with dense elliptical nuclei. During the sixth day, the serosa cells secrete on their periphery a non-chitinous (Campbell, 1929) membrane called the yellow cuticle (Jahn, 1935a, 1935b, and 1936). It is usually complete by the beginning of the seventh day at 25° C. Jahn (1936) found this thin membrane ($< 1\,\mu$) to show a high degree of ionic impermeability, and it may be closely related chemically to the cuticulin of *Rhodnius prolixus* (Wigglesworth, 1933).

The serosa cells also secrete a white fibrous membrane differing structurally and chemically from the yellow cuticle, and lying just inside of it. This layer, which gave Campbell and Jahn a positive chitosan test, is the white cuticle. Slifer's (1937) microscopical examination showed it "to be composed of innumerable fine threads tangled closely together." The deposition of this layered membrane (about 20 μ thick) requires one week at 25° C. (Slifer, 1937). She concluded, "The yel-

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[2] Aided by grant from the National Health Institute, administered by Professor J. H. Bodine.

low layer confers a high degree of impermeability; while the white layer is responsible for a greatly increased toughness and resistance to mechanical injury."

As the embryo develops, the chorion, if allowed to dry, cracks into an irregular pattern. The yellow cuticle is broken during the hatching process, but the embryo apparently is not strong enough to break the tough fibers of the white cuticle. Just before hatching, the latter, or the major portion of it, is digested away, making possible the emergence of the nymph. Slifer (1937, 1938b) has submitted evidence that the enzyme which is responsible for this digestion is produced by the pleuropodia.

The investigation of these membranes with the electron microscope requires special techniques and much patience. Since the electron beam is capable of penetrating tissue only about 1 μ in thickness, clear photographs of structures can only be made if the thickness is kept below 0.5 μ. There are many ways proposed for the preparing of extremely thin sections. All methods thus far noted in the literature fall into two categories: *A.* High speed microtomes, or *B.* Variations in the mechanics of sectioning.

Among the high speed microtomes which have been used and recommended are the "Cyclone Microtome" of O'Brien and McKinley (1943) and the two models by Fullam and Gessler (1946). One of the most difficult problems of the high speed microtome seems to be the locating of the sections after they are cut.

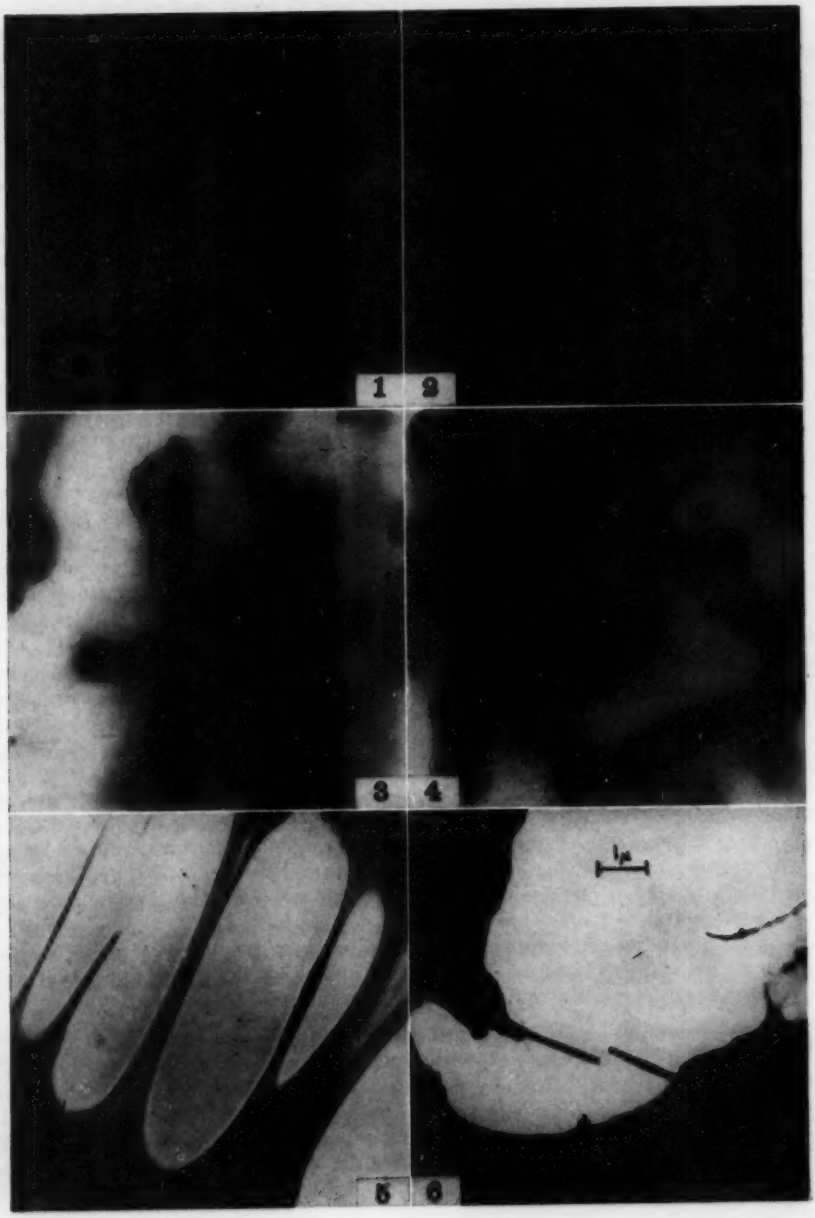
The earliest workers with the electron microscope tried many variations of geometry, mechanics, and chance. One of the methods, which involves a great deal of chance, is to cut the thinnest sections possible on the conventional microtome. These sections are re-embedded and re-sectioned, the operator hoping to obtain at least one very thin section out of many original cuts. The most commonly tried method, and the method used in this study, was the cutting of wedge-shaped sections on the conventional microtome as described by von Ardenne (1939), and Richards, Anderson and Hance (1942). From these a great many fine electronmicrographs have been produced. A very successful method used by Pease and Baker (1948) is the modification of the conventional Spencer Model 820 rotary microtome by decreasing the pitch of the diagonal backing plate to enable the cutting of 0.1 μ thick sections from doubly embedded material.

Another method of preparing sections, which has been used by Richards and his associates at the University of Minnesota, is to choose material which is naturally very thin. The results of these studies are reported by Richards and Anderson (1942), Anderson and Richards (1942), Richards and Korda (1947), and Richards and Korda (1948). The present investigator has employed this method in securing electronmicrographs of the yellow cuticle.

All membranes used were obtained from the eggs of animals kept under laboratory conditions as described by Boell (1935). To obtain the extremely thin sections of the membranes the following methods were used:

A. Fresh yellow cuticle. As stated above, the yellow cuticle is deposited by the serosa cells about the sixth day after the eggs are laid. Six-day-old eggs were placed in sodium hypochlorite solution to dissolve off the chorion (Slifer, 1945). The only membrane left enclosing the egg at this age is the yellow cuticle. The yellow cuticle of the egg was ruptured in isotonic saline solution, and a piece of it (after rinsing in isotonic saline solution and distilled water) is placed on the object screen of the electron microscope.

PLATE I



B. Preserved sections of chorion and white cuticle.

1. Geometrical method. One end of eggs at different stages of development was cut off, and the eggs fixed in Bouin and embedded in paraffin (56° MP) by the usual method. A number of eggs were embedded parallel in each paraffin block. The blocks were sectioned longitudinally or thereabouts in the conventional microtome at settings from 2–10 μ (Dempster, 1942). Some of the above eggs were prepared leaving yolk and embryo in the membranes, and some were prepared with the yolk and embryo removed before fixing. Little difference was noted in the results. The only sections having wedge characteristics were those cut from the eggs at the beginning and ending of the sectioning. These sections were placed in xylol to dissolve out the paraffin before being mounted on the object screen of the electron microscope.
2. Modifying Spencer Model 820 Rotary Microtome. With this modification, eggs were doubly embedded as described by Pease and Baker (1948).

The placing of the sectioned material upon the object screen or grid near the center is not without problems. Since the grid wires are opaque, they always obscure a part of the material from view.

Since the limit of adjustment of the holder is only about five meshes of the grid in diameter, the exact centering of the specimen is critical. In sections carefully prepared and mounted, it may turn out that the material to be observed will have a location behind a wire of the grid. The super-drying of the specimen in the vacuum chamber, plus the "baking" it receives from the electron beams, renders the material so fragile and brittle that its relocation on the grid is next to impossible.

Results

An examination of the electronmicrographs with their titles and explanations reveals the structure of the membranes of the grasshopper egg. These figures are presented from the many pictures taken, as typical of the materials examined. In general, as the eggs become older, the chorion and yellow cuticle become electronically more dense. Thinner sections of older membranes were necessary before the material could be viewed or the electronmicrographs taken.

Figures 1 and 2 of the chorion (using wedge-shaped preserved sections) indicate that it appears to have no clearly resolvable internal structure. The shades of gray of the electronmicrographs vary greatly with the thickness of the sections. Figure 1 is a rather thick section of chorion, while Figure 2 shows a thinner section, and the additional thickness of torn yellow cuticle is at the edge.

Plate I

Figure 1. At edge of chorion from 14 days postdiapause eggs. Preserved specimen. × 13,000.

Figure 2. Chorion from 14 days postdiapause eggs. Torn edge of white cuticle at top. Preserved specimen. × 13,000.

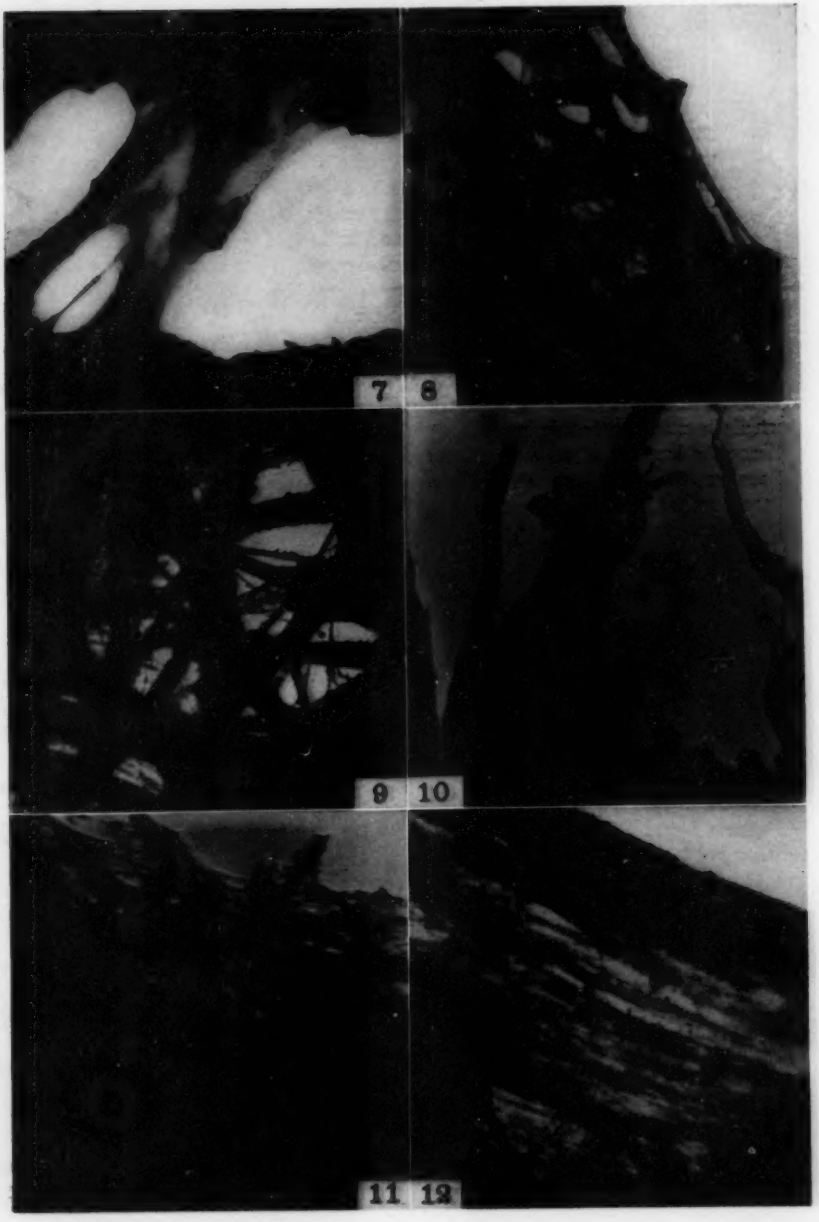
Figure 3. Yellow cuticle from 6-day-old eggs. Fresh specimen. × 13,000.

Figure 4. Same as Figure 3.

Figure 5. Stretched yellow cuticle from 6-day-old eggs. Fresh specimen. × 13,000.

Figure 6. Stretched yellow cuticle from 16 days postdiapause eggs. Preserved specimen. × 13,000.

PLATE II



Since wedge-shaped sections were used, no exact measurements of thickness were possible. All estimates or comparisons of thickness were the result of noting the distance from the thin edge of the wedge. Cross sections of preserved chorion, when stained with Delafield's hematoxylin and eosin or with Mallory's triple stain, appear under the high power oil immersion lens of the light compound microscope to be composed internally of short uneven fibers (Slifer, 1937, 1938a). However, the unstained, longitudinal, wedge-shaped sections of the chorion examined in the electron microscope seem to reveal no indications of a fiber-like structure. (See Figs. 1 and 2.)

Figures 3 and 4 of the yellow cuticle give evidence of the "minute ridges and tubercles" as described by Slifer (1937). These appear on the outer surface of this membrane. The ridges give greater thickness to the yellow cuticle and are believed to be responsible for the "Dalmatian-dog" pattern, which appears to be larger in the fresh material (Figs. 3 and 4) than in the preserved specimens (Figs. 13 and 14). This difference in size may be due to variations in material and shrinkage of the preserved material.

Figure 5 shows the results of stretching the fresh yellow cuticle which occurs as it dries in the electron microscope. Even in these stretched strands, variations in thickness are apparent. Figures 5 and 6 show preserved yellow cuticle which has been pulled to the point of breaking. Figures 5 and 6 both seem to indicate that there is stretching before the strands break. Note the blunt ends of the broken strands.

Figures 8 through 12 show the fibrous layered structure of the white cuticle. It was discovered that if the wedge-shaped sections were stained in eosin before being mounted on the grid in the electron microscope, the fibrous structure was largely obliterated. Since this was interpreted as an artifact, all stains were omitted on membranes employed in this study.

Decided differences of structure between the yellow and white cuticles, as evidenced in this and previous studies, point to the serosa cells as embryonic in nature and differing biochemically during development. The ability of the serosa cells to secrete two different structures or membranes, the yellow and the white cuticle within the same egg, indicates a similarity of function to the epidermal cells of insects (Wigglesworth, 1948).

Figures 8 and 9 show an extreme variation in the size of the fibers. There are indications of individual fibers and bundles of fibers both being present in the same white cuticle. No explanation is offered for the nodules on the fibers which are

PLATE II

FIGURE 7. Stretched yellow cuticle appearing at crack in chorion from 16 days postdiapause eggs. Preserved specimen. × 13,000.

FIGURE 8. White cuticle fibers from 11 days postdiapause eggs. Preserved specimen. × 13,000.

FIGURE 9. White cuticle fibers from 11 days postdiapause eggs. Preserved specimen. × 13,000.

FIGURE 10. Stretched white cuticle fibers from 11 days postdiapause eggs. Preserved specimen. × 22,200.

FIGURE 11. White cuticle fibers from 11 days postdiapause eggs. Preserved specimen. × 13,000.

FIGURE 12. White cuticle fibers from 11 days postdiapause eggs. Preserved specimen. × 13,000.

PLATE III

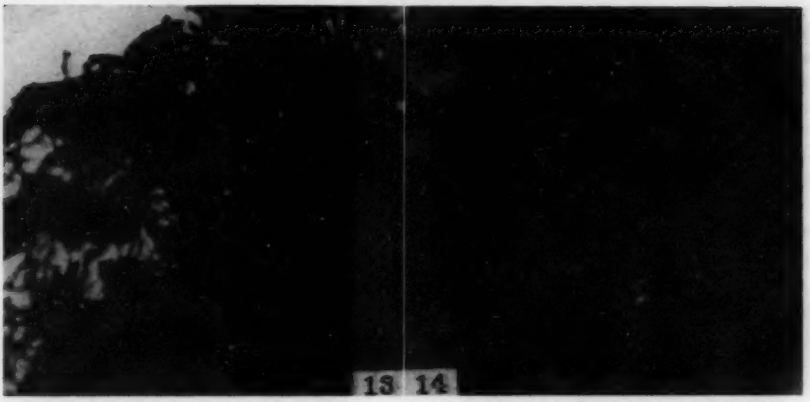


FIGURE 13. Yellow and white cuticle from 11 days postdiapause eggs. Preserved specimen. × 13,000.

FIGURE 14. Yellow and white cuticle from 11 days postdiapause eggs. Preserved specimen. × 13,000.

especially noticeable in Figure 9. Figure 10 shows the results of stretching white cuticle.

Figures 13 and 14 are combinations of yellow and white cuticle taken near the boundary of the two tissues. These electronmicrographs illustrate again the close proximity of the two cuticles, as parts of each may be viewed in one thin section. The "Dalmatian-dog" pattern is smaller in figures from preserved specimens than in Figures 3 and 4 from fresh yellow cuticle.

SUMMARY

Electronmicrographs of the grasshopper egg membranes by the methods used show that:

A. There is no clearly resolvable internal structure of the chorion.
B. The yellow cuticle has no clearly resolvable internal structure, but has varying thicknesses due to minute ridges or projections on its outer surface.
C. The white cuticle is fibrous in structure.

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PHOSPHATASES IN NORMAL AND REORGANIZING STENTORS

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The histochemical demonstration of phosphatase activity in *Stentor coeruleus*, although desirable in its own right, was undertaken primarily to determine to what extent such a study might amplify data obtained earlier (Weisz, 1949) with regard to metabolism and differentiation during normal and reorganizational stages in the life cycle of Stentor. Enzyme activity was studied in the normal, vegetative animal to provide a frame of reference, and this is compared with analogous data on starvation, regeneration, physiological reorganization, and vegetative division.

Experimental

In Stentor, phosphatases cannot be demonstrated with the usual "alkaline" techniques (e.g., Lillie, 1948). Air-dried (cf. below) or acetone-fixed test slides always yield precipitates in the same regions and in the same intensity as control slides prepared by omitting incubation or by substituting a calcium nitrate incubation for the treatment with substrate. Acid phosphatase activity, on the other hand, can be visualized.

The technique was adapted from Gomori's (1941) original method to demonstrate acid phosphatase activity. Stentors of appropriate stages were put on slides with a minimum of water, and after the organisms had been oriented, all excess water was drained off and the preparations were dried in a gentle air current from a fan. This method simultaneously flattens the animal to section thinness and fastens it to the glass. The preparations were then treated directly with the substrate.

In initial exploratory tests the usual method of fixation was tried by dropping living Stentors into chilled acetone before treating them with the substrate. It was found, however, that with this procedure much of the subsequent impregnation potential is lost, possibly due to partial enzyme inactivation by acetone (Stafford and Atkinson, 1948), possibly also due to a leaching out of some of the enzyme in the washing process (Barthelmez and Bensley, 1947). Air-drying on the other hand gave maximal and consistent results.

Of a number of substrates tried, sodium glycerophosphate was found to give the best results (cf. also Gomori, 1949), and this substrate, buffered to pH 4.7, and allowed to act for 15 to 20 hours at 25° C., was consequently used routinely. Post-incubation treatment followed the sulfide technique as outlined by Gomori. Control tests were carried out both by omitting incubation, and by poisoning the substrate with sodium fluoride (M/1000).

Recent work has raised some doubt whether the precipitates obtained by this method represent correct visualizations of phosphatase, and whether the loci of the precipitates correspond precisely to the *in vivo* sites of enzyme activity. Non-enzymatic impregnation of certain tissues by lead salts is known to occur (Lassek,

1947), especially during long incubation. The extent of this error can be estimated, however, by running controls in poisoned substrates. Inasmuch as in Stentor such control preparations do not reveal any precipitate, non-enzymatic impregnation probably does not occur to any appreciable extent under the present conditions of testing; the lead sulfide deposits obtained in the experimental material may thus be regarded presumptively as visualizations of enzyme activity. Nevertheless, in view of the possibility of enzyme shifting during the testing procedure (Barthelmez and Bensley, 1947), caution is warranted in interpreting the results, both with respect to the specificity and the localization of the reaction.

Examination of about 50 Stentors has shown that lead deposits are always found in a number of definite, circumscribed regions. In the ectoplasm, the deposits are centered in the basal granules of the body cilia and the membranelles. This gives the impression that the entire gullet and the peristome band are heavily impregnated, and that the longitudinal rows of body cilia are underscored with dark brown deposits. In the endoplasm, precipitates are particularly constant and abundant around the macronuclear nodes, but no deposits are observed within the nodes themselves. Heavy deposits are also found on the surface and probably within the endoplasmic vacuolar fat reserves (cf. Weisz, 1949), as well as in the immediate vicinity of the gastrioles. (In contrast, preparations fixed in acetone before incubation reveal only light deposits in the circumnuclear site, and no other part of the organism is impregnated.)

In starvation, a gradual decrease in the phosphatase reaction becomes manifest. Deposits in the fat vacuoles and near the gastrioles disappear first. By the time the oral area is about to be resorbed, only the regions around the macronuclei, and the basal granules of the membranelles, still yield a faint reaction (at this stage the preparations resemble those of normal animals which had been fixed in acetone). After the degeneration of the oral area even the circumnuclear activity soon disappears, and no part of the animal reveals any lead sulfide deposits. The data for physiological reorganization and vegetative division are rather parallel, and may be discussed together. The normal sites of activity largely persist unchanged throughout both types of reorganization. The point of interest centers around the regions in which new peristome bands are differentiated, anteriorly in physiological reorganization, and at mid-body in division (cf. Weisz, 1949). In every case, as in normal membranelles, newly differentiated membranelles show a high degree of activity in their basal granules. Such activity, however, can never be observed before the membranelles themselves have formed and are functional. In areas adjacent to newly formed peristome sections, i.e., in areas in which new membranelles will appear within a short time, activity is not yet evident.

Tests carried out on regenerating posterior fragments afford another opportunity to check on this point. Since the time at which new membranelles appear in a Stentor fragment is known (Weisz, 1948), it is possible to test for phosphatase before as well as after peristome new-formation. Such paired tests can be carried out on fragments obtained from the same animal. This was done, with results as above: presumptive sites of newly differentiating membranelles do not reveal any deposits; the latter become manifest only when the membranelles themselves can first be seen in an active state.

Apart from these differences in the presumptive oral area, regenerating frag-

ments do not differ from normal intact animals in the extent and the localization of the sulfide deposits.

If the deposits may indeed be regarded as visualizations of acid phosphatase activity, these observations tend to throw some light on the function of the enzymes in the basal granules of the membranelles and the body cilia, even if only in a negative sense: if the enzyme were to appear just prior to membranelle formation, a role concerned with the mechanics of ciliary differentiation and structural maintenance might be tentatively ascribed to the enzyme. Since this, however, is not the case, the enzyme may possibly be involved in the energetics of ciliary motion.

In summary, the results tend to show that phosphatases in Stentor are fairly consistently present at definite loci of the cytosome, and that reorganization processes, unless they lead to the death of an animal or a fragment, are not correlated with significant changes in enzyme distribution. Newly differentiating organelles manifest characteristic enzymatic activity in parallel with morphological differentiation as such. The presence of phosphatases at circumnuclear sites may be significant in view of evidence (Weisz, 1949) that the macronuclear nodes discharge secretions (possibly phosphate-containing nucleic acid derivatives) into the endoplasm.

Summary

Phosphatase activity is studied in *Stentor coeruleus* by means of histochemical methods. "Alkaline" procedures are negative. Acid phosphatase may be consistently demonstrated in normal Stentors around the macronuclei, in the basal granules of the membranelles and the body cilia, in the endoplasmic fat vacuoles, and around the gastrioles. During starvation a gradual decrease in intensity and distribution of enzyme activity is observed, while in regeneration, physiological reorganization, and in vegetative division, activity remains unaltered in comparison to the normal animal. Presumptive evidence is obtained indicating that acid phosphatase in the basal granules is not primarily a factor in ciliary differentiation.

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THE PROTHORACIC GLANDS OF INSECTS IN RETROSPECT AND IN PROSPECT

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In 1762 the French anatomist, Lyonet, described as "granulated vessels" a pair of minute organs located within the thorax of caterpillars. This description was soon forgotten and for 187 years has been buried among the literature pertaining to insect anatomy. Meanwhile, within the twentieth century, the very same organs have been, and apparently continue to be, rediscovered by various investigators.

The resurrection of Lyonet's description of the "granulated vessels" seems particularly appropriate at the present time. For, within the past ten years, it has become increasingly evident that these organs, now known as "prothoracic glands," are among the most important endocrine glands in insects. The following remarkable paragraphs, which are quoted in translation, are therefore worthy of note, since they present not only the first, but to this day, the most complete description of the gross morphology of the new endocrine organs.

LYONET, P., 1762. Traité Anatomique de la Chenille Qui Ronge le Bois de Saule. Pages 435–437.

THE GRANULATED VESSELS

"Before going on to examine the fat-body and the parts which it encases, there remains to be described two strange vessels, which, on account of their small size, we did not mention in the general outline which we have given in Chapter VI on the interior parts of the caterpillar.

"These vessels, which, on account of their structure, will be called the granulated vessels, are located on the tracheae on the posterior side of the prothoracic spiracle where they form a semi-circle around trachea *a*. They pass dorsally along the tracheal branches *b* and *c*, the muscle *d*, and the cephalic tracheae, *e* and *f*. Each ends on its respective side between the cephalic tracheae *f* and *g*.

"They were consistently present in all the caterpillars of this species which I examined. On account of their small size they are difficult to distinguish easily without a magnifying glass, and they may be mistaken at first for a section of fat. When viewed through the microscope, they appear as they are represented in Pl. XII Fig. 8; in other words, like a long, narrow, irregular and curved mass of longish adherent grains of varying sizes, usually smaller in the direction of the superior line and at the points of insertion of the nerves and tracheae.

"When this mass of grains is dissected, it is found to be formed by a long membranous sac loaded with small blisters which open on it and which are filled, as is the sac, with white matter which presents nothing specific.

Nerves. "In the subject from which Fig. 8 was taken, the nerves A,A,A,A, which supply the granulated vessels seemed to me to come from the third and fourth branch and from the second subdivision of the second branch of the last pair of nerves from the second ganglion; those marked B,B, from the second pair of nerves from the third ganglion; and those marked C,C,C, from the first spinal connectives.

Tracheae. "The tracheae which insert here seemed to me to come from the first and from the second cephalic trunks, but I have neglected to examine, as should have been done, the tracheae and nerves of this small section.

Function. "I have not been able to discover the purpose of the granulated vessels. The relationship they have to the ovaries of some insects could cause them to be taken for real ovaries.

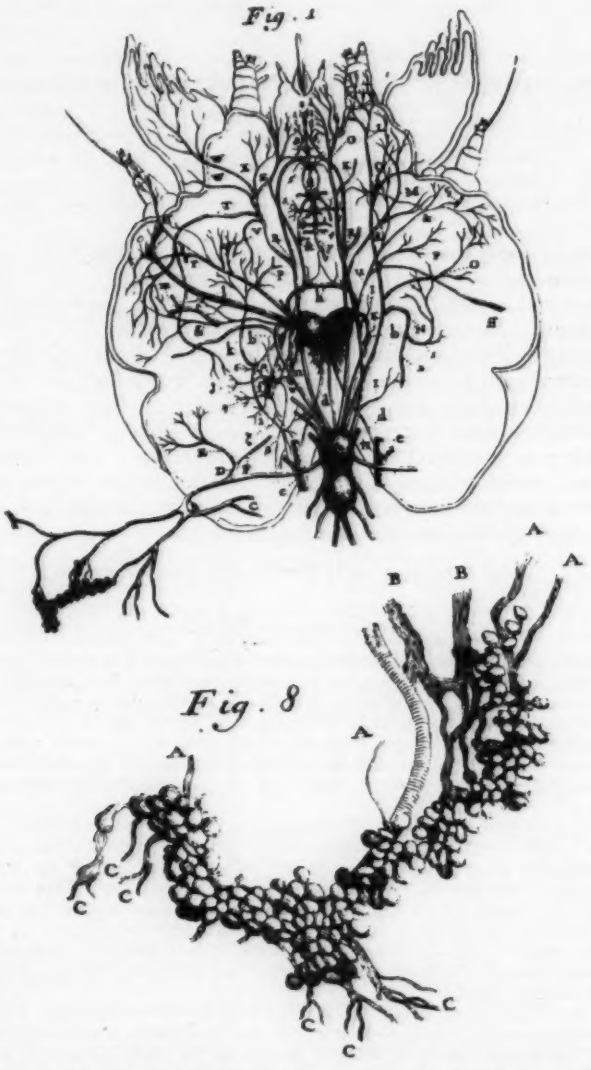

FIGURE 1. From Lyonet's Plate XII. Dissection of the head of the caterpillar as seen in ventral view. Note the prothoracic gland attached to its nerves in the lower left hand corner of the figure.

FIGURE 8. From Lyonet's Plate XVIII. Enlarged view of a prothoracic gland, showing innervation and tracheal supply.

But their location and form, which are very different from that of the ovary of the moth of this caterpillar, indicate the contrary. One might possibly suspect that they are the rudiments of the wings of this animal. This idea occurred to me first; but I was soon corrected when, upon opening a caterpillar on the point of changing into a pupa, I distinctly recognized the wings of the moth, and did not fail to find also the granulated vessels which had not changed shape. It is therefore only through studies of the anatomy of the pupa or the moth that one may hope to discover something on this point."

Nearly two centuries elapsed before application was made of the advice of the final paragraph. Then, in a series of investigations reported in 1940, 1941, and 1944, Fukuda was able to demonstrate that the prothoracic glands of *Bombyx mori* were involved in the endocrinological control of moulting, pupation, and adult development.

In the past few years certain of Fukuda's observations have been confirmed on other insects. In the Cecropia silkworm, for example, the development of the adult moth within the pupa requires a hormone secreted by the prothoracic glands (Williams, 1947, 1949a). But, in addition to this factor, the initiation of adult development also requires the *presence* of a second hormone secreted by the brain (Williams, 1946, 1947, 1949b) and the *absence* of a third hormone secreted by the corpora allata (Williams, 1949c).

Evidence of this type makes it increasingly clear that neither the prothoracic glands nor any other organ are endowed with the complete control over metamorphosis. In insects as in mammals, hormonal mechanisms seem to require a certain complexity in order to prove feasible and self-balancing.

From these considerations we are led to a somewhat more moderate evaluation of the role of the prothoracic glands than that which Fukuda has proposed. The prothoracic glands are but one component in an endocrinological system that presides over metamorphosis: among the other components in this system must be included at least two further organs, the brain and the corpora allata.

Notwithstanding this complication, the identification of the prothoracic glands of Lepidoptera as endocrine organs has provoked a re-examination of the endocrinology of metamorphosis and a search for comparable organs in other orders of insects.

The glands are now known to occur, not only in the Lepidoptera (Lee, 1948), but also in the Hymenoptera (Williams, 1948). Apparently homologous organs have been described as "prothoracic glands" in the Orthoptera (Scharrer, 1948), as "intersegmental organs" in the Odonata (Cazal, 1947, 1948; Deroux-Stralla, 1948a), and as "ventral glands" in certain other hemimetabolous insects (Pflugfelder, 1947). In the case of the Odonata the anatomical affinity between intersegmental organs and prothoracic glands has been reinforced by experimental evidence. Thus, according to Deroux-Stralla (1948b), the removal of the intersegmental organs results in abnormalities in moulting and suppression of adult development—effects that would be anticipated in terms of the proposed homology.

Since our knowledge of insect endocrinology is based so largely on studies of the Hemiptera, it is significant that organs anatomically identical with prothoracic glands have been found in nymphs of the lygaeid, *Oncopeltus fasciatus* (Edwards, 1948).

Of further interest is the description by Possompès (1946) of a pair of "peritracheal glands" in larvae of Chironomus. Possompès calls attention to the histo-

logical resemblance between these peritracheal glands in the lower Diptera and the lateral cells in the ring-gland of the higher Diptera. If the ring-gland should prove to contain a component homologous to the prothoracic glands of other insects, it would account for some of the peculiar endocrinological effects of this organ.

Thus, notwithstanding the fragmentary character of the present evidence, it seems probable that prothoracic glands and their homologues are widely distributed among insects. A more detailed evaluation of their role in development may lead to a sounder understanding of metamorphosis and may open to experimental attack certain facets of the problem that have hitherto remained inaccessible. In accordance with the advice of Lyonet, it is only through further study that we may hope to discover something on this point.

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